FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

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Synthesis of biodiesel from wastes of food industry via direct transesterification with methanol/carbon dioxide mixtures

By
Filipe Miguel de Oliveira Calixto

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Supervisor: Professor Doutor Pedro Miguel Calado Simões
Co-ordinator: Professora Doutora Susana Filipe Barreiros

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"A person who never made a mistake never tried anything new"

Albert Einstein (1879-1955)

Abstract

Direct transesterification with supercritical methanol of spent coffee ground oil in the temperature range of 523 to 603 K and in the pressure range of 15 to 30 MPa has been studied, and fatty acid methyl esters yield up to 85% were obtained. The percentage of oil contained in spent coffee grounds has been determined by soxhlet extraction with ethanol, resulting a value of 14% in oil.

Carbon dioxide as co-solvent was added to methanol with the aim of decreasing the operating temperature and pressure. In order to determine the optimal conditions of the various factors that influence the transesterification with CO_2 in a minimum number of experiments, the design of experiments (DOE) was applied. It was demonstrated that, at a reaction temperature of 573 K, pressure of 10 MPa and a molar ratio CO_2/MeOH of 0.1 (optimal operation conditions), a 93% yield of methyl esters was obtained. The percentage in C16 (methyl palmitate) and C18 (methyl stearate, methyl oleate and methyl linoleate) has been determined in all samples. The C16 and C18 yields obtained in the experiment at 573 K, 10 MPa and a molar ratio CO_2/MeOH of 10% were of 41.5% and 52%, respectively.

Keywords: Biodiesel; Carbon dioxide; Extraction; Methanol; Methyl Esters; Transesterification; Supercritical fluids.

Resumo

Neste trabalho experimental estudou-se a transesterificação directa da borra de café com metanol supercrítico na gama de temperaturas entre 523 e 603 K e na gama de pressões entre 15 e 30 MPa, obtendo-se um rendimento máximo de ésteres metílicos de 85%. Foi também determinada a percentagem de óleo contida na borra de café através da extracção por etanol em soxhlet, resultando no valor de 14% em óleo.

Com a finalidade de fazer diminuir a temperatura e pressão de operação, adicionou-se ao metanol dióxido de carbono como co-solvente. Aplicou-se o desenho de experiências com o intuito de optimizar os parâmetros que influenciam a transesterificação com CO_2, num número mínimo de experiências. Foi demonstrado que à temperatura de reacção de 573 K, pressão de 10 MPa e na razão molar CO_2/MeOH de 0.1 (condições óptimas de operação), o rendimento em ésteres metílicos foi de 93%. As percentagens de C16 (palmitato metílico) e de C18 (estearato metílico, oleato metílico e linoleato metílico) foram também determinadas em todas as amostras. Na experiência a 573 K, 10 MPa e razão molar CO_2/MeOH de 10%, obteve-se um rendimento de C16 e C18 de 41,5% e 52%, respectivamente.

Palavras Chave: Biodiesel; Dióxido de carbono; Extracção; Metanol; Metil Esteres; Transesterificação; Fluidos Supercríticos.

Contents

Abstract 4
Keywords 4
Resumo 5
Palavras Chave 5
Contents 6
List of Figures 8
List of Tables 11
List of Abbreviations and Symbols 13
Introduction 15
Chapter 1 State of Art 16
1.1 Global energy: Overview 17
1.1.1 Fossil Fuels 17
1.1.2 Renewable Sources 18
1.2 Biodiesel 20
1.3 Transesterification of Vegetable Oils 24
1.3.1 Variables affecting transterification reaction 25
1.4 Supercritical Fluids 28
1.4.1 Supercritical Fluid Extraction 29
1.4.2 Transesterification in Supercritical Fluids 34
1.5 Direct Transesterification of spent coffee grounds oil in supercritical fluids 36
Chapter 2 Materials and Methods 39

2.1 Materials 40

2.1.1 Chemicals 40

2.1.2 Equipment 41

2.1.3 Experimental set-up 42

2.2 Methods 44

2.2.1 Sample preparation and characterization 44

2.2.2 Direct Transesterification of spent coffee grounds oil in supercritical methanol 45

2.2.3 Direct Transesterification of spent coffee grounds oil in supercritical methanol/CO_2 mixture 45

2.2.4 Evaporation of methanol in the sample 46

2.3 Sample analysis 46

Chapter 3 Results and Discussion 48

3.1 Direct transesterification in supercritical methanol 49

3.2 Direct transesterification from spent coffee grounds oil using supercritical methanol and CO_2 as co-solvent 53

Chapter 4 Conclusions and Scope for Future Works 66

Chapter 5 References 69

Appendix 78

Appendix A – Experimental results 79

Appendix B – ANOVA table of the design of the experiences 82

Appendix C – Replication of the best level 84

Appendix D – Calibration curves 85

Appendix E – Molar Ratios CO_2/MeOH 87

List of Figures

Figure 1.1 – Annual Energy consumption values for selected countries 18
Figure 1.2 – Percent reduction in emissions of pollutants in B100 and B20. TH is total hydrocarbons, CO_2 is carbon dioxide, CO is carbon monoxide, PM is particulate matter, NO_x is nitrogen oxides, PHA is polycyclic hydrocarbons, and NPAH is nitrated PAHs. 21
Figure 1.3 – World edible oil production from the four most important oilseeds: rape, oil palm, soya and sunflower. 22
Figure 1.4- Overall transesterification reaction. 24
Figure 1.5 - Changes in yield percentage of methyl esters at different temperatures as a function of reaction time. Molar ratio of vegetable oil to methyl alcohol: 1:41. Sample: hazelnut kernel oil. 27
Figure 1.6 - Definition of supercritical state for a pure component. CP critical point, TP triple point, Tc critical temperature, Pc critical Pressure 28
Figure 2.1 – Apparatus for direct transesterification of spent coffee grounds oil in supercritical methanol and supercritical methanol/CO_2 mixture..... 42
Figure 2.2 – Soxhlet Extractor..... 44
Figure 2.3 – Saturated fatty acid methyl ester: methyl palmitate. 47
Figure 2.4 – Unsaturated fatty acid methyl ester: methyl oleate. 47
Figure 3.1 – Synthesis of spent coffee ground oil methyl esters, at various temperatures and pressures in supercritical methanol. ◆: 523K and 15 MPa; ■: 553 K and 20 MPa; ▲: 583 K and 25 MPa; ●: 603 K and 30 MPa. Methyl flow rate was 0.5 ml/min. 49
Figure 3.2 – Yield of Triglycerides extracted but not converted in supercritical transesterification with methanol for different conditions of pressure and temperature. 50
Figure 3.3 – Yield of methyl esters converted from triglycerides in supercritical methanol for different conditions of pressure and temperature..... 51
Figure 3.4 – Mass Fractions of polar and non-polar phases for several samples in direct transesterification with supercritical methanol. 52

Figure 3.5 – Synthesis of spent coffee ground oil methyl esters, at various temperatures and pressures in supercritical methanol and CO_2 as co-solvent. Molar ratio CO2/MeOH was fixed at 10%. ●: 473 K and 10 MPa; ◇: 473 K and 20 MPa; ▲:573 K and 10 MPa; □:573 K and 20MPa. 55

Figure 3.6 – Synthesis of spent coffee ground oil methyl esters, at various temperatures and pressures in supercritical methanol and CO_2 as co-solvent. Molar ratio CO2/MeOH was fixed at 30%. ●: 473 K and 10 MPa; ◇: 473 K and 20 MPa; ▲: 573 K and 10 MPa; □:573 K and 20 MPa. 55

Figure 3.7 – Synthesis of spent coffee ground oil methyl esters, at various temperatures and ratios CO_2/MeOH in supercritical methanol and CO_2 as co-solvent. Pressure was fixed at 10MPa. ●: 473 K and 10%CO_2; ◇: 473 K and 30%CO_2; ▲:573 K and 10%CO_2; □:573 K and 30%CO_2. 56

Figure 3.8 – Synthesis of spent coffee ground oil methyl esters, at various temperatures and ratios CO_2/MeOH in supercritical methanol and CO_2 as co-solvent. Pressure was fixed at 20MPa.: ●:473K and 10%CO_2; ◇:473K and 30%CO_2; ▲: 573K and 10%CO_2; □: 573K and 30%CO_2. 56

Figure 3.9 – Synthesis of spent coffee ground oil methyl esters, at various pressures and ratios CO_2/MeOH in supercritical methanol and CO_2 as co-solvent. Temperature was fixed at 473 K. ●: 10% CO_2 and 10MPa; ◇:10%CO_2 and 20MPa; ▲:30%CO_2 and 10MPa; □: 30%CO_2 and 20MPa. 57

Figure 3.10 - Synthesis of spent coffee ground oil methyl esters, at various pressures and ratios CO_2/MeOH in supercritical methanol and CO_2 as co-solvent. Temperature was fixed at 573 K. ●: 10% CO_2 and 10MPa; ◇:10%CO_2 and 20MPa; ▲:30%CO_2 and 10MPa; □: 30%CO_2 and 20MPa. 57

Figure 3.11 - Yield of Triglycerides extracted but not converted in supercritical transesterification with methanol and CO_2 as co-solvent in different condition of pressure and temperature. 59

Figure 3.12 – Yield of methyl esters converted from triglycerides in supercritical transesterification with methanol and CO_2 as co-solvent in different condition of pressure and temperature. 59

Figure 3.13 – Changes in GC chromatograms of the product as a function of reaction time in direct transesterification with supercritical methanol and co-solvent (molar ratio

CO_2/MeOH is 0.1) at 573 K and 10 MPa. C16, methyl palmitate; C18 methyl stearate, methyl oleate and methyl linoleate. 60

Figure 3.14 – Mass Fraction of polar and non-polar part for several samples in direct transesterification with supercritical methanol and CO_2 as co-solvent. 61

Figure 3.15 – Percentage of methyl esters estimated by STATISTICA7 software, varying the pressure and the ratio CO_2/MeOH, at a constant temperature of 523 K. 62

Figure 3.16 – Percentage of methyl esters estimated by STATISTICA7 software, varying the temperature and the ratio CO_2/MeOH, at a constant pressure of 15MPa.... 63

Figure 3.17 – Percentage of methyl esters estimated by STATISTICA7 software, varying the pressure and the temperature, at a constant ratio CO_2/methanol of 20%. ... 63

Figure 3.18 – Methyl Esters yield for the best essay at 573 K, 10 MPa and molar ratio CO_2/MeOH of 10% and two replications. ▲: Experimental test; □: Replica 1; ◇: Replica 2. 64

Figure 3.19 – Synthesis of spent coffee ground oil methyl esters, at various temperatures in supercritical methanol and CO_2 as co-solvent. Carbon dioxide and methanol were taken at a molar ratio of 10% with a constant pressure at 10 MPa. ◇: 473 K; □: 523 K; ▲:573 K. 65

Figure D. I – Calibration curve for Methyl Palmitate (C16) peaks in GC, equation of calculated trend line and its correlation coefficient. A(C16): peak area of Methyl Palmitate. 85

Figure D. II – Calibration curve for Methyl Stearate, Methyl Oleate and Methyl Linoleate (C18) peaks in GC, equation of calculated trend line and its correlation coefficient. A(C18): peak area of Methyl Stearate, Methyl Oleate and Methyl Linoleate. 85

Figure D. III – Calibration curve for Tripalmitolein peaks in GC, equation of calculated trend line and its correlation coefficient. A(Triglycerides): peak area of Tripalmitolein. 86

List of Tables

Table 1.1 – Fatty acid distribution of some vegetable oils 23
Table 1.2 – Order of magnitude of physical properties (density, diffusion and viscosity) for gaseous, supercritical and liquid state..... 29
Table 1.3 – Processing Parameters in the Extraction of Solids 33
Table 1.4 – Critical Temperatures and critical pressures of various alcohols 35
Table 1.5 – Comparison between catalytic methanol (MeOH) process and supercritical methanol (SCM) method for biodiesel from vegetable oils by transesterification 35
Table 1.6 – Oil content of "traditional" vegetable oils and spent coffee ground..... 37
Table 1.7 – Comparison of Coffee Oil with Other Waste Feedstock for Biodiesel Production..... 37
Table 2.1 – All compounds used in this experimental work 40
Table 3.1 - Parameters studied to optimize of the direct transesterification of spent coffee ground oil using supercritical methanol and CO_2 as co-solvent, according to the DOE..... 53
Table 3.2 - Design of experiments (DOE) of transesterification from spent coffee grounds oil using supercritical methanol and CO_2 as co-solvent..... 54
Table 3.3 – Yield of methyl esters for the best level and two replications for a molar ratio CO_2/methanol of 10%, temperature of 573 K and pressure of 10 MPa. 64

Table A. I - Experimental data for direct transesterification of spent coffee grounds oil with supercritical methanol. 79
Table A. II - Methyl Esters formed and triglycerides extracted mass in supercritical transesterification with methanol in different condition of pressure and temperature. .. 79
Table A. III - Experimental data for direct transesterification of spent coffee grounds oil with supercritical methanol and CO_2 as co-solvent at a molar ratio CO_2/methanol of the 10%. 80

Table A. IV - Experimental data for direct transesterification of spent coffee grounds oil with supercritical methanol and CO_2 as co-solvent at a molar ratio CO_2/methanol of the 30%. 80
Table A. V – Methyl Esters formed and triglycerides extracted percentage in supercritical transesterification with methanol and CO_2 as co-solvent in different condition of pressure and temperature, at a molar ratio CO_2/methanol of 10 %..... 81
Table A. VI - Methyl Esters formed and triglycerides extracted percentage in supercritical transesterification with methanol and CO_2 as co-solvent in different condition of pressure and temperature, at a molar ratio CO_2/methanol of 30 %..... 81

Table B. I – ANOVA table given by STATISTICA7 software. 82
Table B. II – F Cumulative Probabilities: 95% confidence level ($\alpha = 0.05$)..... 83

Table C I – Yield of methyl esters for the best level and its two replications. Molar ratio CO_2/methanol of 10%, temperature of 573K and pressure of 10MPa..... 84

Table E I – Isobaric Properties for Methanol at 10 MPa..... 87
Table E II – Isobaric Properties for CO_2 at 10MPa[1] 87
Table E III – Isobaric Properties for Methanol at 20 MPa[1] 87
Table E IV – Isobaric Properties for CO_2 at 20MPa 88

List of Abbreviations and Symbols

B20 – 20 percent biodiesel and 80 percent petroleum

B100 – 100 percent biodiesel

BPR – Back Pressure Regulator

C16 – Methyl Palmitate

C18 - Methyl Stearate, Methyl Oleate and Methyl Linoleate

CO_2 – Carbone dioxide

FAAE – Fatty Acid Alkyl Esters

FAME – Fatty Acid Methyl Esters

FFA – Free Fatty Acid

FI – Flux Indicator

GC – Gas Chromatography

HPLC - High-performance liquid chromatography

K - Kelvin

KOH – Potassium hydroxide

KOMe - Potassium methoxide

min. – minute

MeOH – Methanol

MPa – Mega Pascal

NA – Not Available

NaOH – Sodium hydroxide

NaOMe - Sodium methoxide

NG – Natural Gas

P_C – Critical pressure

PI – Pressure Indicator

SC – Supercritical

SC-CO_2 – Supercritical carbon dioxide;

SCG – Spent Coffee Ground

SCM – Supercritical methanol

T_C – Critical temperature

TI – Temperature Indicator

Wt% - weight percent

ν – Degree of freedom

ρ – Density

Introduction

In recent times, the world has confronted the twin crises of fossil fuel depletion and environmental degradation. The indiscriminate exploration and consumption of fossil fuels has led to a reduction in petroleum reserves. Alternative fuels, energy conservation and management, energy efficiency and environmental protection have become important in recent years. From the point of view of global environment protection and the concern for long-term supplies of conventional diesel fuels, it becomes necessary to develop alternative fuels comparable with conventional fuels. Alternative fuels should be, not only sustainable but also environmentally friendly. A good diesel oil substitute is biodiesel

The work aimed to optimize the operating parameters affecting direct transesterification of spent coffee grounds in supercritical methanol with and without carbon dioxide as co-solvent. The source of spent coffee ground was the bar of the Chemistry Department building at FCT campus.

Initially, it made up the preparation and characterization of spent coffee grounds oil. Then it was optimized the temperature and pressure of direct transesterification with supercritical methanol. For this purpose some tests were made.

However, the synthesis of biodiesel by supercritical methanol has the drawback of using high temperature and pressure, which are not viable in industry. Thus, carbon dioxide was added into the reaction mixture with the aim of decreasing the operating temperature and pressure. In order to determine the optimal conditions of the various factors such as temperature, pressure and molar ratio of CO_2/MeOH, in a minimum number of experiments, the design of experiments was applied.

Final conclusions and some suggestions for future work are outlined in the final phase of the study.

Chapter 1 State of Art

1.1 Global energy: Overview

1.1.1 Fossil Fuels

The motivation behind the development and use of alternative fuels are mainly, the diminishing reserves of readily recoverable oil from fossil origin, the concern over global climate change, the increasing fuel prices and the desire for energy independence and security (Ueno, et al. 2001).

The major part of all the energy consumed worldwide comes from fossil like coal, petroleum and natural gas (NG), with the exception of hydroelectricity and nuclear energy, all these sources are finite and at the current usage rates will be consumed shortly. Petroleum-based fuels have limited reserves concentrated in specific regions of the world. These sources are in the verge of getting extinguished (A. Demirbas 2007).

Diesel fuels have an essential function in the industrial economy of a developing country and are used for transport of industrial and agricultural goods and operation of diesel tractor and pump sets in the agricultural sector. Economic growth is always accompanied by a commensurate increase in transports (Meher, Sagar and Naik 2004).

Figure 1.1 shows that, between 1985 and 2005, annual energy consumption increased 31 percent in the United States, while only 18 percent in Europe, and an overwhelming 250 percent in China and India.

Currently available fossil fuel sources are estimated to become nearly depleted within the next century, with petroleum fuel reserves depleted within 40 years. The maximum rate of oil production is expected to occur between 2010 and 2020 (Drapcho, Nhuam and Walker 2008). Thus, the high energy demand in the industrialized world as well as in the domestic sector and pollution problems caused due to the widespread use of fossil fuels make it increasingly necessary to develop renewable energy sources of limitless duration and small environmental impact than the traditional one (Korbitz 1999).

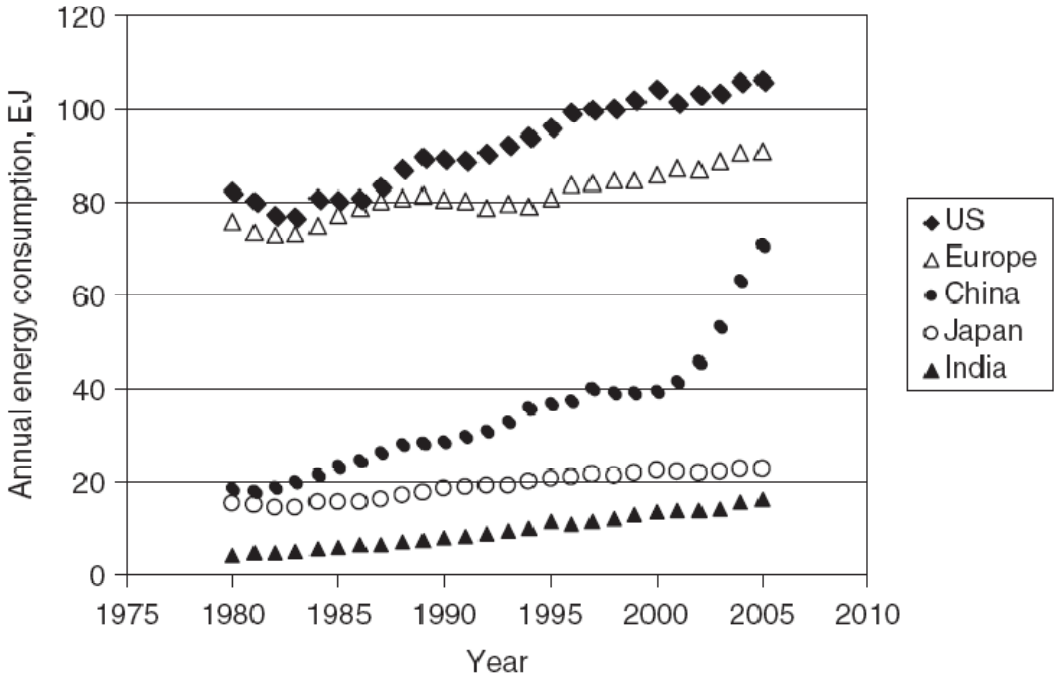

Figure 1.1 – Annual Energy consumption values for selected countries[1]

1.1.2 Renewable Sources

Renewable energy deriving from solar, wind, and biomass sources has great potential for growth to meet our future energy needs. Alternative new and renewable fuels have the potential to solve many of the current social problems and concerns, from air pollution and global warming to other environmental improvements and sustainability issues (Urbanchuk 2003).

There is a growing interest in the valorization of waste and by-products from agrochemical industries, not only by using them as alternative raw materials for different processes, but also as a means to reduce their environmental impact. The European Commission is taking measures to ensure that the target of 20 % renewable energy share of final energy consumption in 2020 will be met (European Commission 2008).

[1] Adapted from Energy Information Agency, 2007.

Fuels such as ethanol, hydrogen, or biodiesel are characterized as biofuels because they can be produced by the activity of biological organisms and they can be used in the energy, food, and nutraceutical/pharmaceutical industries (Drapcho, Nhuam and Walker 2008). Biofuels are generally considered as offering many advantages, including sustainability, reduction of greenhouse gas emissions, regional development, social structure and security of supply (Reijnders 2006).

Biofuel production, if approached in a sustainable manner, can be more environmentally benign than fossil fuel technologies for several major reasons. First, biofuel production from biomass is largely carbon neutral –that is, the CO_2 produced as the fuel is combusted, is offset by the carbon absorbed as the biomass is grown (Tillman, Hill and Lehman 2006). Second, bioconversion processes in general do not produce hazardous compounds, and if toxic solvents and chemicals are avoided in the processing stages, then fewer environmental pollutants are produced. Third, biomass production and microbial conversion processes can be developed and used in a more distributed manner, avoiding the need for transport of fuels via cargo ships or pipelines for long distances (Drapcho, Nhuam and Walker 2008).

In developed countries there is a growing trend towards employing modern technologies and efficient bioenergy conversion using a range of biofuels, which are becoming cost-wise competitive with fossil fuels (Puhan, et al. 2005).

The scarcity of conventional fossil fuels, growing emissions of combustion generated pollutants and their increasing costs will make biomass sources more attractive (Sensoz, Angin and Yorgun 2000). On the other hand, biomass use, in which many people already have an interest, has the properties of being a biomass source and a carbon neutral source (Dowaki, et al. 2007).

From the point of view of protecting the global environment and the concern for long-term supplies of conventional diesel fuels, it becomes necessary to develop alternative fuels comparable with conventional fuels, therefore alternative fuels should be environmentally friendly.

1.2 Biodiesel

Recently, the world has been confronted with the twin crises of fossil fuel depletion and environmental degradation, particularly global warming (Sheehan, et al. 1998).A potential diesel oil substitute is biodiesel. Biodiesel consists of methyl esters of vegetable oils or animal fats and is produced by the transesterification reaction of triglycerides with short-chain alcohols, mainly methanol. The resulting biodiesel is quite similar to conventional diesel fuel in terms of their main characteristics (Meher, Vidya Sagar and Naik 2006).

Biodiesel has a viscosity similar to conventional petroleum diesel, moreover it is non-inflammable, and in contrast to petroleum diesel it is non-explosive, with a flash point of 423 K for biodiesel as compared to 337 K to petroleum diesel. Unlike petrodiesel, biodiesel is biodegradable and non-toxic, and it significantly reduces toxic and other emissions when burned as a fuel (A. Demirbas 2009).

Emissions resulting from the use of biodiesel in combustion engines are greatly reduced compared to conventional petroleum diesel fuels by up to 100 percent sulfur dioxide, 48 percent carbon monoxide, 47 percent particulate matter, 67 percent total unburned hydrocarbons, and up to 90 percent reduction in mutagenicity (Lotero 2005). In Figure1.2, it is shown the emission reductions for biodiesel B100 (100 % biodiesel) and B20 (20% biodiesel blend) compared to conventional petroleum diesel fuels.

Biodiesel is renewable and does not contribute to global warming due to the closed carbon cycle. A life cycle analysis of biodiesel showed that overall CO_2 emissions were reduced by 78% compared with petroleum-based diesel fuel, which is considered the most important greenhouse gas in climatic models (Sheehan, et al. 1998).

A variety of feedstocks can be used to produce biodiesel and these feedstocks can be divided into first and second generation feedstocks. First generation feedstocks refer to vegetable food oils like rape seed, palm, soybean or sunflower oil. However, second generation feedstocks consist of non-edible seed oils such as Jatropha curcas or castor oil and waste oils (A. Demirbas 2006).

World annual petroleum consumption and vegetable oil production is about 4.018 and 0.107 billion tons, respectively. Global vegetable oil production increased from 56 million tons in 1990 to 88 million tons in 2000, following a below normal increase (A. Demirbas 2008).

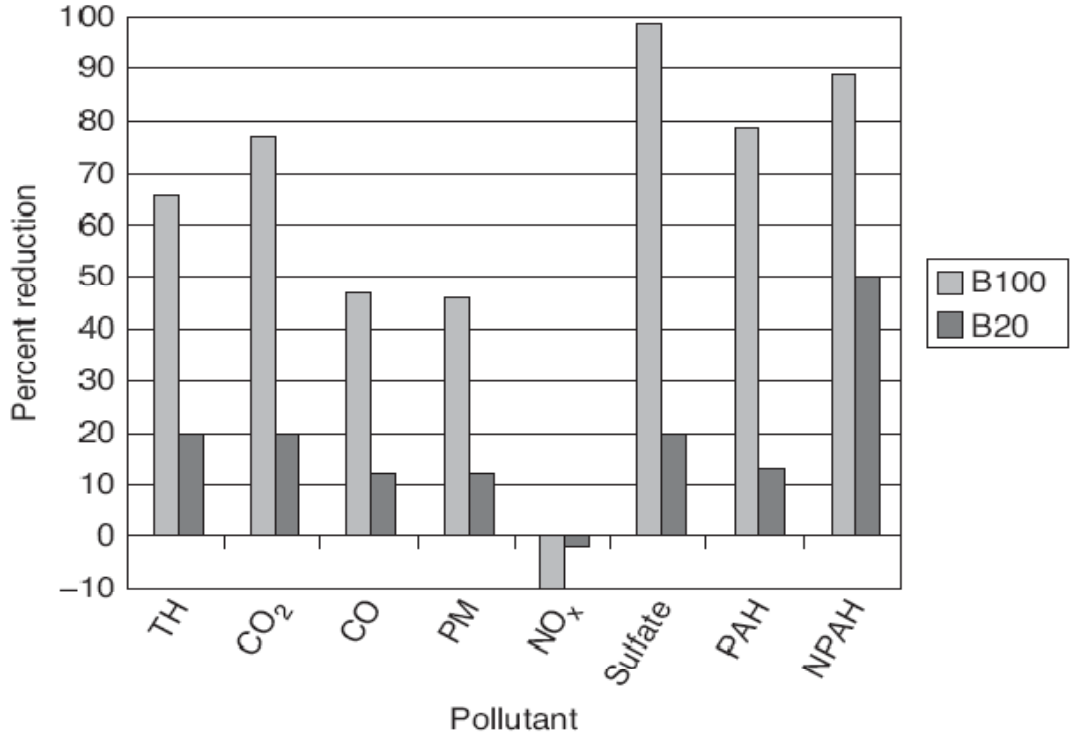

Figure 1.2 – Percent reduction in emissions of pollutants in B100 and B20. TH is total hydrocarbons, CO_2 is carbon dioxide, CO is carbon monoxide, PM is particulate matter, NO_x is nitrogen oxides, PHA is polycyclic hydrocarbons, and NPAH is nitrated PAHs. [1]

Chemically, oils and fats consist among other lipids of triglycerides, which are glycerides that are esterified with three fatty acids. These fatty acids may differ by the length of carbon chains, the number, orientation and position of double bonds in these chains (Meher, Vidya Sagar and Naik 2006).

Several types of vegetable oils can be used for the preparation of biodiesel. Figure 1.3 shows the world edible oil production from the four most important oilseeds: rape, oil palm, soya and sunflower. The fatty acid compositions of some common vegetable oils, and also of coffee oil, which was used as the feedstock for the production of biodiesel in this work, are given in Table 1.1.

[1] (Adapted from http://www.biodiesel.org/pdf_files/fuelfactsheets/emissions.pdf.)

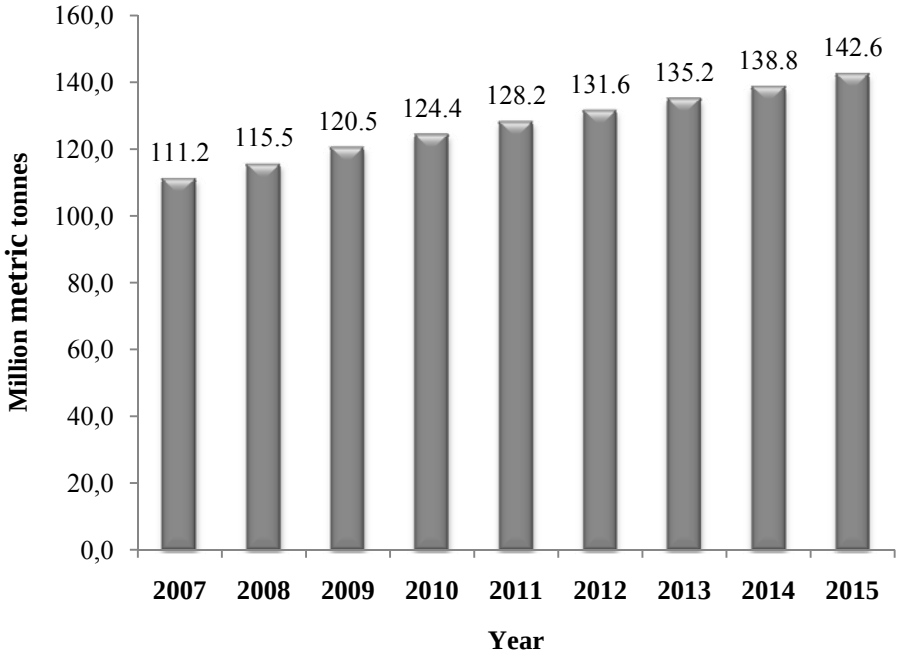

Figure 1.3 – World edible oil production from the four most important oilseeds: rape, oil palm, soya and sunflower.[1]

The advantages of vegetable oils as diesel fuels are liquid nature-portability, higher heat content (about 88% of diesel fuel), their renewability, lower sulfur and aromatic contents and ready availability and biodegradability (A. Demirbas 2005).

Disadvantages of using vegetable oils for biodiesel are their relative lower energy content (8 % less than petroleum diesel), higher viscosity, lower volatility, reactivity of unsaturated hydrocarbons (oxidative stability) (A. Demirbas 2003).

Vegetable oil has too high a viscosity too high to be used in most existing Diesel engines as a straight replacement fuel oil. There are a number of ways to reduce the viscosity of the vegetable oil. Dilution, micro-emulsification, pyrolysis and transesterification are the four techniques applied to solve the problems encountered with the high fuel viscosity. One the most common methods to reduce oil viscosity in the biodiesel industry is called transesterification (Pinnarat and Savage 2008)

[1] Source: (Heinrich 2009)

Table 1.1 – Fatty acid distribution of some vegetable oils

Vegetable-based oil	Fatty acid distribution (% by weight)							Ref.
	Palmitic 16:0	Palmitoleic 16:1	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Others	
Rape (canola) oil	3.5	-	0.9	64.4	22.3	8.2	0.7	[1]
Olive oil	9.2	0.8	3.4	80.4	4.5	0.6	1.1	[1]
Sunflower oil	6.0	-	4.2	18.7	69.3	-	1.8	[1]
Safflower oil	5.2	-	2.2	76.3	16.2	-	0.1	[1]
Soybean oil	10.6	-	4.8	22.5	52.3	8.2	1.6	[1]
Palm oil	47.9	-	4.2	37.0	9.1	0.3	1.5	[1]
Cottonseed oil	28.7	-	0.9	13.0	57.4	-	-	[2]
Poppy seed oil	12.6	0.1	4.0	22.3	60.2	0.5	0.3	[2]
Sesame seed oil	13.1	-	3.9	52.8	30.2	-	-	[2]
Linseed (flax) oil	5.1	0.3	2.5	18.9	18.1	55.1	-	[2]
Wheat grain oil	20.6	1.0	1.1	16.6	56.0	2.9	1.8	[2]
Corn oil	11.8	-	2.0	24.8	61.3	-	0.1	[2]
Peanut oil	11.4	-	2.4	48.3	32.0	0.9	5	[2]
Hazelnut oil	4.9	0.2	2.6	83.6	8.5	0.2	-	[2]
Walnut oil	7.2	0.2	1.9	18.5	56.0	16.2	-	[2]
Almond oil	6.5	0.5	1.4	70.7	20.0	-	0.9	[2]
Jatropha curcas	13.3	1.0	4.9	32.0	45.0	0.2	3.6	[3]
Rice bran oil	21.5	-	2.9	38.4	34.4	2.2	0.6	[4]
[5]Coffee oil	38.7	0.7	7.5	9.0	35.9	3.1	5.1	[6]

[1] Source: (Lotero 2005)
[2]Source: (A. Demirbas 2003)
[3] Source: (Tiwari, Kumar and Raheman 2007)
[4]Source: (Xu and Godber 1999)
[5] Green Coffee Arabica oil
[6]Source: (Al Kanhal 1997)

1.3 Transesterification of Vegetable Oils

Vegetable oils are made of triglycerides molecules, in which three fatty acid groups are esters attached to one glycerol molecule. The triglyceride molecules differ by the nature of the alkyl chain bound to glycerol. Transesterification is the process of reacting triglycerides with an alcohol in presence of a catalyst (e.g. a strong acid or a base) to produce a mixture of fatty acid alkyl esters (FAAE) and glycerol. It consists of a sequence of three consecutive reversible reactions where triglycerides are sequentially converted to diglycerides and these to monoglycerides and finally conversion of monoglycerides to FAAE and glycerol (Meher, Sagar and Naik 2004). Figure1.4 shows the overall reaction.

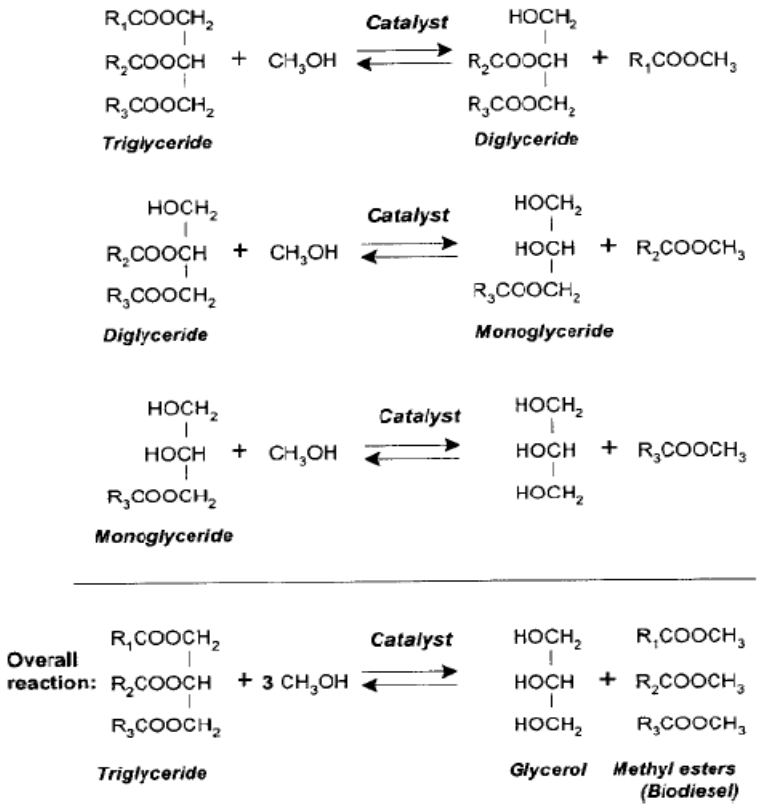

Figure 1.4- Overall transesterification reaction[1].

[1]Source: (Pinnarat and Savage 2008)

The stoichiometric reaction requires 1 mol of a triglyceride and 3 moles of the alcohol, however, an excess of the alcohol is used to increase the yields of the alkyl esters and to allow its phase separation from the glycerol formed.

The transesterification reaction proceeds using primary or secondary monohydric aliphatic alcohols. Among them the most used in this reaction are methanol, ethanol, propanol, butanol and amyl alcohol. Methanol and ethanol are used frequently, but methanol is preferable because of its low cost and its physical and chemical advantages (polar and shortest chain alcohol) (A. Demirbas 2005). The transesterification reaction can be catalyzed by alkalis (Ramadhas, Jayaraj and Muraleedharan 2004), acids (Furuta, Matsuhashi and Arata 2004), or enzymes (Noureddini, Gao and Philkana 2005).

Acids used for transesterification include methanolic sulfur acid (Sahoo, et al. 2007), ferric sulfate (Wang, et al. 2007), methanolic hydrogen chloride (Darnoko and Cheryan 2000), methanolic boron trifluoride (Rule 1997) and sulfonic acid (Guerreiro, et al. 2006). Transesterification by acid catalysis is much slower than by alkali catalysis (A. Demirbas 2009).

Alkalis used for transesterification include NaOH or KOH (A. Demirbas 2009), Demirbas reported that the alkali-catalyzed transesterification proceeds much faster than that catalyzed by the same amount of an acidic catalyst and thus it is most often used commercially.

The enzyme-catalyzed transesterification processes are not yet commercially developed, new results have been reported in some articles (Shah, Sharma and Gupta 2004) (Noureddini, Gao and Philkana 2005) (Du, et al. 2004). The common aspects of these studies consist in optimizing the reaction conditions (solvent, temperature, pH, etc) in order to establish suitable characteristics for an industrial application. However, the reaction yields as well as the reaction times are still unfavorable compared to the base-catalyzed reaction systems (A. Demirbas 2008).

1.3.1 Variables affecting transterification reaction

The process of transesterification is affected by various factors depending upon the reaction condition used. The effects of these factors are described below.

1.3.1.1 Effect of water and free fatty acids (FFA)

The free fatty acid moisture content are key parameters for determining the viability of the vegetable oil transesterification process. To carry the base catalyzed reaction to completion; an free fatty acid value lower than 3% is needed. The higher the acidity of the oil, smaller is the conversion efficiency. Both, excess as well as insufficient amount of catalyst may cause soap formation (Meher, Sagar and Naik 2004). The resulting soaps can induce an increase in viscosity, formation of gels and foams, and make the separation of glycerol difficult (Ghadge and Raheman 2005).

The effects of free fatty acids and water on transesterification of beef tallow with methanol were investigated (Ma, Clements and Hanna 1998). The results showed that the water content of beef tallow should be kept below 0.06% w/w and FFA content of beef tallow should be kept below 0.5% w/w in order to get the best conversion.

Water content is an important factor in the conventional catalytic transesterification of vegetable oil. Free fatty acids and water always produce negative effects since the presence of FFA and water causes soap formation, consumes catalyst, and reduces catalyst effectiveness (A. Demirbas 2006).

1.3.1.2 Effect of molar ratio

The molar ratio of alcohol to oil is one of the most important variables influencing the conversion of oil into alkyl esters, because excess alcohol shifts the reaction to product side. Ramadhas *et al*. (Ramadhas, Jayaraj and Muraleedharan 2004) and Sahoo et al. (Sahoo, et al. 2007), have reported a 6:1 molar ratio for acid esterification and a 9:1 molar ratio for alkaline esterification as the optimum values for biodiesel production from high FFA rubber seed oil and polanga seed oil, respectively. Veljkovic et al. (Veljkovic, et al. 2006) have achieved an 18:1 molar ratio during acid esterification and 6:1 molar ratio during alkaline esterification. Meher *et al*. (Meher, Vidya Sagar and Naik 2006) have taken 6:1 molar ratio during acid esterification and 12:1 molar ratio during alkaline esterification.

Higher molar ratios result in higher ester production in a shorter time. Vegetable oils are transesterified with 1:6-1:40 vegetable oil-alcohol molar ratios in catalytic conditions (A. Demirbas 2002).

1.3.1.3 Effect of reaction time and temperature

The conversion rate increases with reaction time. Ma et al. (Ma and Hanna 1999) studied the effect of reaction time on transesterification of beef tallow with methanol. The reaction was very slow during the first minute due to mixing and dispersion of methanol into beef tallow. From one to 5 min, the reaction proceeds very fast. The production of beef tallow methyl esters reached the maximum value about 15 min.

Transesterification can occur at different temperatures, depending on the oil used. For the transesterification of refined oil with methanol (6:1) and 1% NaOH, the reaction was studied at three different temperatures. After 0.1 h, ester yield were 9, 87 and 64% for 60, 45 and 32 ºC, respectively. After 1h, ester formation was identical for 60 and 45 ºC runs and only slightly lower for the 32 ºC run. Temperature clearly influenced the reaction rate and yield of esters (Ma and Hanna 1999).

Figure 1.5 shows a typical example of the relationship between the reaction time and the temperature (A. Demirbas 2009). As it can be seen, increasing the reaction temperature has a favorable influence on the yield of ester conversion.

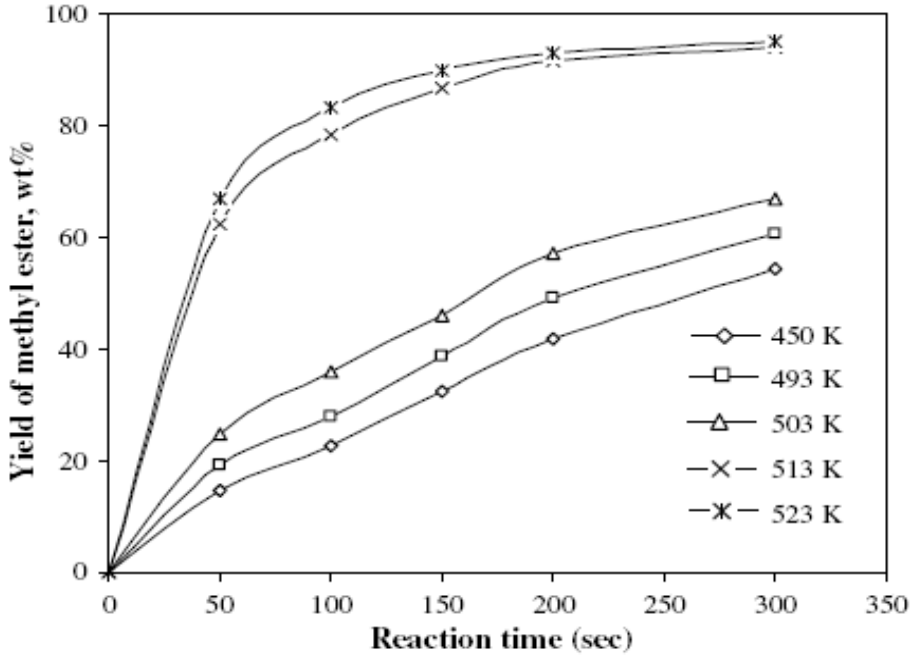

Figure 1.5 - Changes in yield percentage of methyl esters at different temperatures as a function of reaction time. Molar ratio of vegetable oil to methyl alcohol: 1:41. Sample: hazelnut kernel oil.

1.4 Supercritical Fluids

The supercritical state is achieved, when the temperature and pressure of a substance is raised over its critical values (critical temperature and critical pressure). The critical point represents the highest temperature and pressure at which the substance can exist as a vapor and liquid in equilibrium. This can be explained with reference to the phase diagram for pure component (Figure 1.6). The liquid becomes less dense because of thermal expansion and gas becomes denser as the pressure rises. Eventually the densities of the two phases become identical at the critical point. The fluid above this state is described as a supercritical fluid. In the supercritical state the distinction between the liquid and the gas phase has disappeared and liquid can no longer be liquefied by raising the pressure nor can gas be formed by increasing the temperature (Kamat, Beckman and Russel 1995).

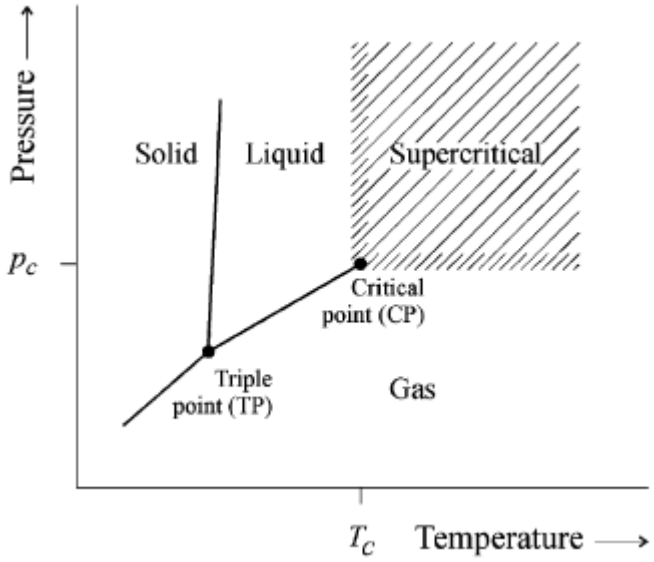

Figure 1.6 - Definition of supercritical state for a pure component. CP critical point, TP triple point, Tc critical temperature, Pc critical Pressure[1]

Under supercritical conditions, the properties of the substance (e.g. density, viscosity, diffusion coefficient…) are intermediate between those of liquid and gas. The density is typically of the order of magnitude of liquid density (from 0.1 to 0.8 g cm^{-3}). The solvating power of supercritical fluids is also very similar to the one of many conventional organic solvents and much higher than in gases. Moreover, the diffusion

[1] (Brunner 1994)

coefficient of supercritical fluids is about 5 to 50 times higher than in liquids. This is illustrated in Table 1.2 (Brunner 2005).

Table 1.2 – Order of magnitude of physical properties (density, diffusion and viscosity) for gaseous, supercritical and liquid state.

	Density ($g\ cm^{-3}$)	Diffusion ($cm^2\ s^{-1}$)	Viscosity ($g\ cm^{-1}\ s^{-1}$)
Gas	10^{-3}	10^{-1}	10^{-4}
Supercritical fluid	$10^{-1} – 1$ *Liquid-like*	$10^{-4} – 10^{-3}$ *Liquid-like*	$10^{-4} – 10^{-3}$ *Gas-like*
Liquid	1	$<10^{-5}$	10^{-2}

1.4.1Supercritical Fluid Extraction

Supercritical Fluid Extraction (SFE) is based on the fact that, near the critical point of the solvent, its properties change rapidly with only slight variations of pressure.

Supercritical fluids, as supercritical carbon dioxide, can be used to extract analytes from samples. The main advantages of using supercritical carbon dioxide for extractions is that it is inexpensive, extract the analytes faster and more environmentally friendly than organic solvents. For these reasons CO_2 is the reagent widely used as supercritical solvent (Kondamudi, Mohapatra and Mistra 2008).

Indeed, supercritical fluid extraction (SFE) has immediate advantages over traditional extraction techniques:

1. SCFs have solvating powers similar to liquid organic solvents, but with higher diffusivities, lower viscosity, and lower surface tension;
2. Since the solvating power can be adjusted by changing the pressure or temperature ,separation of analytes from solvent is fast and easy;
3. By adding modifiers to a SCF its polarity can be changed for having more selective separation power;
4. Candidate SCFs are generally cheap, simple and many are safe. Disposal costs are much less and in industrial processes;

5. SCF technology requires sensitive process control, which is a challenge. In addition, the phase transition of the mixture of solutes and solvents has to be measured or predicted quite accurately.

Separation in a SFF process can occur in two different ways:

a) Separation by reducing solvent power (Bruno and Fly 1991):

   i. Using an expansion chamber to expand the extraction phase to conditions where the solvent is sub-critical. A two-phase mixture is formed, consisting of the liquid solvent containing the extract and the remaining gaseous phase of the solvent. Extra costs are needed if recycling of the solvent is wanted;
   ii. Coupling the extraction step with a pressure reduction separation, with no phase change. A pressure reduction leads to lower concentrations of the dissolved substances because of the lower density of the SCF. This solution also requires some extra cost due to the recompression of the solvent;
   iii. To reduce cost in ii, an isobaric heating of the solvent can be used as a separation step. A higher temperature decreases the density of the solvent leading to a lower solvent power. But this increase also increases vapour pressure of the solute, so at low pressures (where density decreases strongly with temperature) it is possible to separate solutes from the SCF; at high pressures (where density decreases moderately with temperature) the opposite occurs and the gas phase is richer in solutes.

b) Separation of extract and solvent by a mass separating agent (Ruivo 2003):
   i. In certain cases the solutes processed into a SFE unit may be advantageously separated from the solvent by absorption. The absorbing liquid should dissolve the solutes not interfering with the quality of them and should not be soluble in the Supercritical solvent. In this case there will be a significant pressure drop on the circuit of the solvent;

ii. Like absorption, the adsorption separation allows to operate the circuit of the supercritical solvent at a constant pressure. Adsorption is very effective with respect to removing the extract from the gas and regenerating the gaseous solvent. This technique can be useful for solutes that do not need to be recovered after the extraction step, since it may be difficult to remove the extract from the adsorbents;

iii. Separation can also be achieved using membrane technology. Membranes allow the operation of the solvent circuit at low-pressure differences.

SFE of solids is the most studied application since the most frequently required separation process is the extraction/elimination of one or more compound families from a solid natural matrix (Reverchon and De Marco 2006). Some examples include the extraction of lipids from cyanobacteria (Mendes, Reis and Palavra 2006), extraction of essential oils from plants (Della Porta, et al. 1999), extraction of lycopene from tomatoes (Cadoni, et al. 2000) and the decaffeination of coffee (Zosel 1978). It has been carried out in a commercial scale for more than two decades, in processes like the decaffeination of coffee beans and black tea leaves and the production of hop extracts (Brunner 1994).

Parameters affecting the supercritical fluid extraction of solids are listed in Table1.3.

The influence of the process parameters can be summarized as follows (Martínez and Vance 2007):

- Solubility of compounds increase by increasing the extraction pressure at constant temperature;
- At pressure close to the critical pressure, solubility of the compounds increases by decreasing the temperature. However, at high pressures, solubility of compounds increases by increasing the temperature. This crossover effect is due to the competing effects of the reduction in solvent density and the increase of the vapour pressure. The latter has marked influence at higher pressure. The pressure at which the crossover effect occurs depends on the type of compounds to extract. The crossover range for most of the compounds takes place between 20 and 35 MPa;

- The separation conditions depend on the solubility of the compounds at different pressures and temperatures as well as whether a fractionation of extract is carried out by sequential depressurization steps. Generally the separation pressure is carried out at 5-6 MPa. For essential oils or volatile fractions, the separation takes place at 3 to 5 MPa and low temperatures to maximize the recovery of the top notes components. For oils, the separation can take place at 15 to 20 MPa due to their low solubility in supercritical carbon dioxide (CO_2) under those conditions;
- The solvent-feed ratio depends on many factors, such as concentration of the solute in the feed material, solubility in the supercritical solvent, type of feed material, and distribution of the compound in the feed material;
- High solvent flow rates imply high operating and capital costs. However, they could increase production capacity. The solvent flow rate or the residence time of the solvent in the extraction vessel must be optimized. A high residence time implies a long batch time. Conversely, a short residence time may result in shorter contact time between the solvent and solute, resulting in a loading of the solvent much lower than the saturation concentration at the selected operating conditions;
- The size and morphology of the solid material have a direct effect on the mass transfer rate. In general, increasing the surface area increases the extraction rate. Therefore, smaller particle size or geometry (such as flakes) generally favors higher mass transfer, decreasing the batch time as well as diffusion controlled process. If the soluble substances are located in rigid structures inside of the solid matrix, the size reduction breaks this structure so it will be easily accessible for the solvent. However, very small particles favour a channelling effect, which decreases the extraction rate. Particle size needs to be evaluated case by case based on the type of material to be processed;
- Similarly to particle size, moisture content must be evaluated case by case. High content of moisture is usually not desirable because moisture acts as a mass transfer barrier. On the other hand, moisture expands the cell structure, facilitating the mass transfer of the solvent and the solute through the solid matrix (e.g. in seeds and beans). For instance, the influence of moisture

between 3% and 10% generally has no significant impact on the mass transfer of edible oil from seeds.

Table 1.3 – Processing Parameters in the Extraction of Solids [1]

Raw Material Related	
• Particle morphology and size • Moisture • Chemical reactions for setting free the extract compounds • Cell destruction • Pelletization	
Operating Conditions	
➢ Extraction conditions: • Pressure • Temperature • Time • Solvent flow • Solvent-feed ratio	➢ Extraction operation: • Fractional extraction • Constant conditions
➢ Separation conditions: • Pressure • Temperature	➢ Separation operation: • Single stage • Fractional separation

The specialty oils rich in bio actives can be extracted from many plant sources. These extracted oils are mainly mixtures of triglycerides, free fatty acids, monoglycerides and diglycerides.

For seed oils, as well as biomass, SC-CO_2 extraction is considered to be a promising alternative to conventional processes of extraction, because SC-CO2 is non-toxic, non-flammable, non-corrosive, cheap, readily available, easily separable and environmental friendly (Sankar and Monohar 2000). Solubility is a strong function of SC-CO_2 density and the properties of the solute, such as molecular weight, polarity, and vapour pressure. All lipid components of interest, especially oils, are soluble in SC-CO_2 to different extents, depending on temperature and pressure conditions. Generally, solubility of lipids in SC-CO_2 decreases with an increase in polarity and molecular

[1] (Martínez and Vance 2007)

weight, thus following the order: fatty acid esters, fatty acids, and triglycerides (Güclü-Üstündag and Temelle 2004).

1.4.2 Transesterification in Supercritical Fluids

Chemical reactions in supercritical fluids are considered as good candidates for new environmentally friendly technologies. The advantages to be gained by conducting reaction in supercritical fluids, apart from the environmental advantages, are as follows. First, there is control of phase behaviour to obtain homogeneity or separation as required. In second place, the increased diffusion can speed up the reactions, both homogeneous and heterogeneous, when diffusion is a controlling factor. Third, there is an enhanced control of reaction conditions, through both pressure and temperature (Afonso and Crespo 2005).

Current commercial production for biodiesel involves the use of alkali-catalyzed method (Khan, Warith and Luk 2007). In this method, however, those oils with high free fatty acid and water contents are difficult to be utilized since the former reacts with alkaline catalyst to form undesirable saponified products and the latter hinders the complete conversion. Furthermore, several purification processes are necessary to remove catalyst and saponified products (Xin, Imahara and Saka 2008). So, the transesterification of triglycerides by supercritical methanol (SCM), ethanol, propanol and butanol has proved to be the most promising process. Table 1.4 shows the critical temperatures and critical pressures of various alcohols.

Transesterification can occur at different temperatures and this variable influences the reaction rate and yield of esters, depending on the oil used. Figure 1.5 shows a typical example of the relationship between the reaction time and temperature. It is observed that increasing the reaction temperature, especially to supercritical temperatures, has a favourable influence on ester conversion (A. Demirbas 2002).

A comparison between the catalytic commercial methanol process and the SCM method for biodiesel from vegetable oils by transesterification is given in Table 1.5. Demirbas (A. Demirbas 2006) has reported a novel method of synthesis of biodiesel fuels from vegetable oil via non-catalytic transesterification in supercritical fluids like methanol, to overcome the problems of conventional methods.

Table 1.4 – Critical Temperatures and critical pressures of various alcohols

Alcohol	Critical temperature (K)	Critical pressure (MPa)
Methanol	512.2	8.1
Ethanol	516.2	6.4
1-Propanol	537.2	5.1
1-Butanol	560.2	4.9

Source: (A. Demirbas 2002)

However, the synthesis of biodiesel by supercritical methanol has the drawback of the high cost of apparatus due to the high temperature and pressure, which are not viable in the large scale practice in industry. So, many researchers have focused on how to decrease the severity of the reaction conditions. Co-solvents, such as carbon dioxide (Han, Cao and Zhang 2005), hexane (Cao, Han and Zhang 2005) and calcium oxide (Dermirbas 2007), added into the reaction mixture can decrease the operating temperature, pressure and the amount of alcohol used (Yin, Xiao and Song 2007).

Table 1.5 – Comparison between catalytic methanol (MeOH) process and supercritical methanol (SCM) method for biodiesel from vegetable oils by transesterification

	Catalytic MeOH process	SCM methanol
Methylating agent	Methanol	Methanol
Catalyst	Alkali	None
Reaction temperature (K)	303-338	523-573
Reaction pressure (MPa)	0.1	10-25
Reaction time (min)	60-360	7-15
Methyl ester yield (wt %)	96	98
Removal for purification	Methanol, catalyst, glycerol, soaps	Methanol
Free fatty acids	Saponified products	Methyl esters, water
Continuity easiness	Not feasible	Easy to implement

Source: (A. Demirbas 2006)

1.5 Direct Transesterification of spent coffee grounds oil in supercritical fluids

Coffee, together with tea, is one of the most popular drinks across the world. Its commercial and social importance is obvious. Coffee production is located mainly in South America, Brazil being the first producing country (42%). Africa and Asia account for 20.4% and 18.5%, respectively, of the total production. Yet, Europe is the main coffee consumer (Martín, et al. 2001).

Europe produces great amounts of agro-industrial residues, such as spent coffee grounds (SCG), which have largely been underexploited, most of them with detrimental effects on the environment. However, what used to be of little concern in the past is now a source of uneasiness, and strategies for tackling environmental issues while creating value are emerging. As with many other agro-industrial residues, SCG are vast resources of lipids, proteins and lignocellulosic materials and their valorization is receiving more attention (Kondamudi, Mohapatra and Mistra 2008). Conversion of lipids into biodiesel is a way to obtain economic profit from these renewable waste resources while contributing to the replacement of fossil fuels.

Spent coffee grounds contain in average up to 20wt% of lipids (87-93% of which are triglycerides), which may be converted into biodiesel, and also high added-value substances, such as tannins, with reported antioxidant and antihypertensive properties, among others (Farah and Donangelo 2006) (Arya and Rao 2007) (Ratnayake 1993) (Kondamudi, Mohapatra and Mistra 2008). According to other studies, it is estimated that 100g of roasted coffee beans originate ca.70g of SCG, on a dry weight basis (Pais 2006).

In Table 1.6 it is observed that spent coffee grounds contain as much oil as "traditional" biodiesel feedstocks, such as rapeseed, palm and soybean oil.

Table 1.6 – Oil content of "traditional" vegetable and spent coffee ground.

Product	Oil Content (% by weight)	Reference
Rapeseed	30	(Lotero 2005)
Sunflower	47	(Lotero 2005)
Soybean	18	(Lotero 2005)
Palm	35	(Lotero 2005)
Spent coffee grounds	11-20	(Kondamudi, Mohapatra and Mistra 2008)

According with some sources, it is estimated that spent coffee grounds can potentially add 340 million gallons per year of biodiesel to the world's fuel supply (Kondamudi, Mohapatra and Mistra 2008). According with these authors biodiesel from coffee grounds would be more stable than those from other sources because coffee contains antioxidants that would slow down degradation. Thus, the quality and cost-benefit of spent coffee ground are better as compared to other waste sources available as seen through Table 1.7.

Table 1.7 – Comparison of Coffee Oil with Other Waste Feedstock for Biodiesel Production.

Source	Amount (million gallons/year)	Advantage	Disadvantage
Animal fat			
Tallow, brown grease, pork fat (white grease), lard, fish oil, poultry fat	5.5[1]	Oxidative stability, less expensive	High sulfur content, high FFA (50-90%), bad odor, bad cold flow properties, high cloud point, high pour point, transportation costs, purification required
Vegetable oil			
Used vegetable oil	2.8[1]	Less expensive, readily available	Bad odor, high FFA
Waste coffee oil	2.92	Less expensive, higher stability, well-established transportation, pleasant smell	Extraction of oil from spent coffee grounds required

Source: (Kondamudi, Mohapatra and Mistra 2008)

[1] (Alleman and McCormick 2008)

Therefore, the present work aimed at an evaluation of the potential of using oil of waste coffee as a feedstock for biodiesel production. For this, it was made the extraction and transesterification of oil in a single step, using methanol and carbon dioxide as co-solvent in supercritical state. Preliminary studies have proven the viability of $ScCO_2$ to extract oil from SCG (Simões, Couto and Fernandes 2009). Additional tests must be done to optimize the main operating conditions of extraction, e.g. solvent pressure/temperature, solvent to biomass mass ratio, extraction time, to maximize extraction yields. These parameters are essential to design a large scale unit and perform an economic evaluation of the process.

Chapter 2 Materials and Methods

2.1Materials

2.1.1Chemicals

The spent coffee ground (brand Tofa) used in all tests was provided by the bar of the Chemistry Department building at FCT campus. Furthermore, the other compounds are described in Table 2.1.

Table 2.1 – All compounds used in this experimental work.

Name	Molecular formula	Molecular mass (g/mol)	% purity	Brand
Methanol for Chromatography	CH_4O	33.04	99.9	Sigma-Aldrich
Hexane for Chromatography	C_6H_{14}	86.18	96	Merck
Carbone Dioxide	CO_2	44.01	99.98	AIR LIQUIDE
Ethylene Glycol	C_2H_6O	62.07	NA	Merck
Squalane	$C_{30}H_{62}$	422.82	99	Aldrich
Methyl Palmitate	$C_{17}H_{34}O_2$	270.46	99	Sigma
Methyl Linoleate	$C_{19}H_{34}O_2$	294.72	99	Fluka
Methyl Stearate	$C_{19}H_{38}O_2$	298.51	96+	Aldrich
Methyl Oleate	$C_{19}H_{36}O_2$	286.49	99	Aldrich
Tripalmitolein	$C_{51}H_{92}O_6$	801.27	99	Aldrich

2.1.2Equipment

The equipment used during the experimental work was the following:

- Temperature and Process Controller CAL3300, model 312-1841;
- Analytical balance METTLER TOLEDO, model PJ12;
- Circulator Bath HAAKE C1-W13;
- PT100 sensor;
- internal thermocouple type K;
- HPLC – PUMP KNAUER, model K-501;
- Back Pressure Regulator (BPR) TESCOM, model 27-1700;
- Gas Chromatography TRACEGC 2000 SERIES;
- Cryostat Julabo, model FT400;
- LCD, Type NSI-33R;
- GC's Oven Carlo Erba Strumentazione, model HRGC 5300 Series;
- Flow controller Parker Pneumatic, model P3D;
- Oven Indelab, model 6882A;
- Heating mantle Barnstead Electrothermal, model EM 1000;
- Rotary evaporator Büchi, model RE111.

2.1.3Experimental set-up

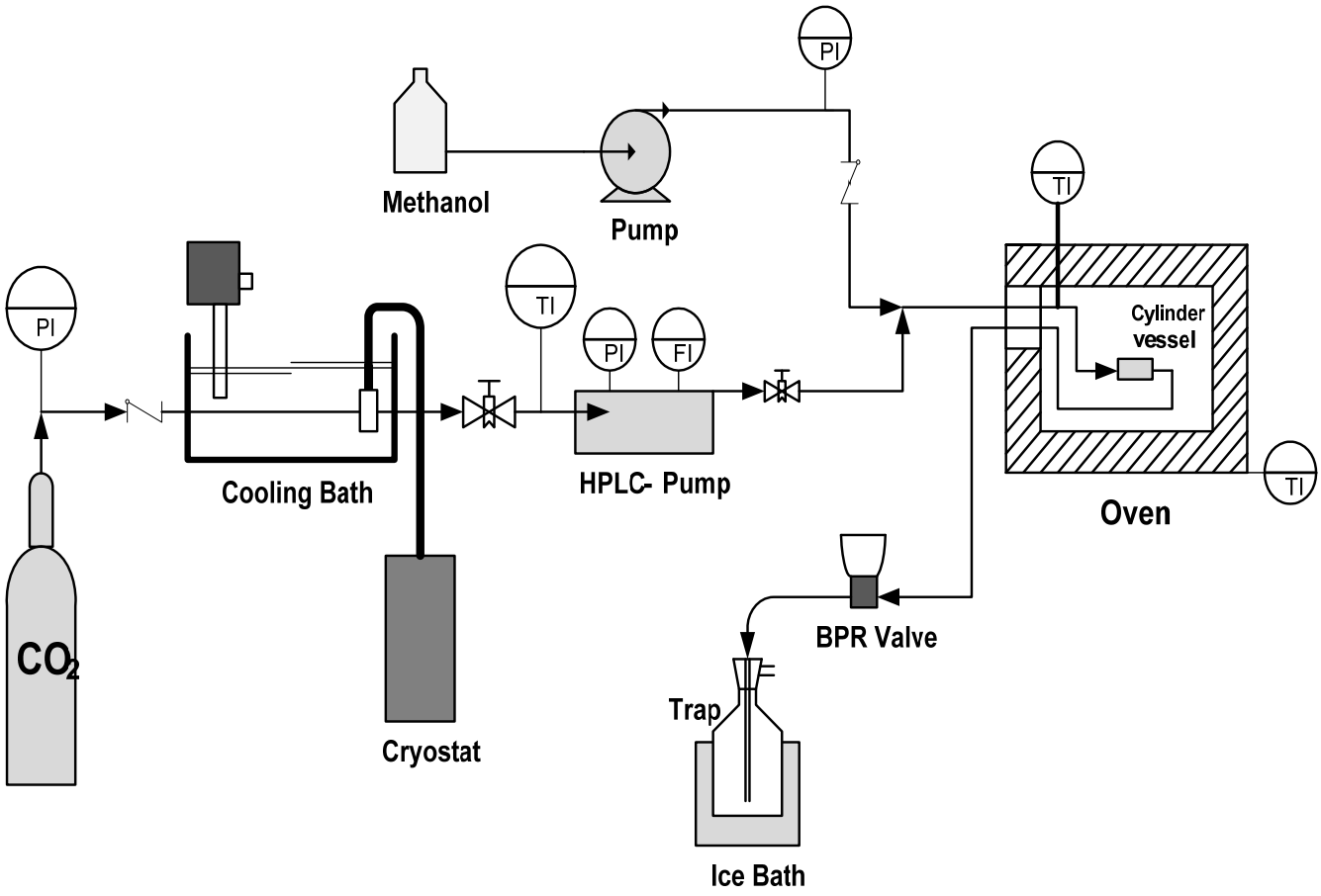

Figure 2.1 – Apparatus for direct transesterification of spent coffee grounds oil in supercritical methanol and supercritical methanol/CO_2 mixture.

The direct transesterification with methanol and methanol/carbon dioxide mixture apparatus is shown in Figure 2.1.

This apparatus is composed of a CO_2 vessel, followed by a tank with water/ethylene glycol (50:50 v/v), where it is immersed a cryostat (Julabo Immersion and Flow-through coolers, model FT400) and a circulator bath (Circulator Bath HAAKE C1-W13) to liquefy the carbon dioxide and allow it to be pumped by an HPLC – Pump (KNAUER, model K-501) through a packed bed reactor. The bath is at a temperature of -10 º C.

The packed bed reactor consists of a cylinder vessel with 10 cm^3 –internal volume where *ca.* 2g of coffee grounds is added. The packed bed reactor is within a

chromatographic oven (Carlo Erba Strumentazione, model HRGC 5300 Series) with temperature indicator and control.

A Back-pressure regulator (BPR) is located at the exit of the oven to control the pressure in the system. Pressure is measured by the HPLC – Pump internal sensor. Temperature is measured at the exit of the oven by an internal PT-100 sensor connected to a digital reader (CAL3300, model 312-1841).

At the end of the installation is placed a glass trap, with the purpose of collecting the formed products and to channel the CO_2 for the exterior. The trap is immersed in an ice bath.

2.2Methods

2.2.1Sample preparation and characterization

Spent coffee grounds, collected from the chemistry department bar were dried at 353 K for five days in an oven (Indelab, model 6882A).

The percentage of oil contained in spent coffee grounds was obtained through soxhlet extraction with ethanol (Figure 2.2). In this method the sample is placed in a porous cellulose thimble. The thimble is placed in an extraction chamber (2), which is suspended above a flask containing the solvent (1) and below a condenser (3). Heating mantle was from Barnstead Electrothermal, model EM 1000. The boiling flask had a capacity of 1000 mL, and the extraction chamber was of 60 mL in volume.

Extraction was performed with ethanol as extraction solvent, and extraction temperature was 352 K (ethanol boiling point). The boiling flask is heated and the ethanol evaporates and moves up into the condenser where it condensed and trickle backs into the extraction chamber containing the sample. The extraction chamber is designed so that when the ethanol surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask.

At the end of the extraction process, the flask containing ethanol and lipid is removed. The ethanol is then evaporated from the extracted oil in a rotary evaporator.

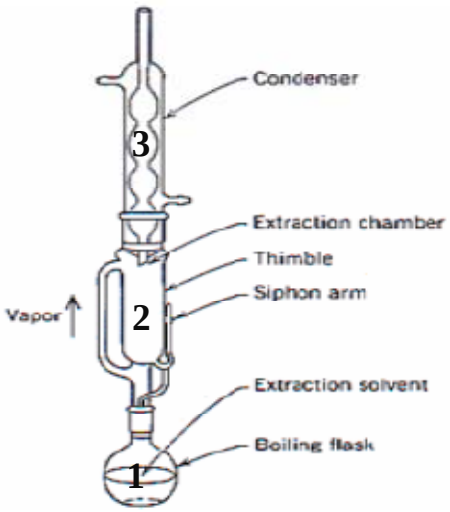

Figure 2.2 – Soxhlet Extractor

2.2.2Direct Transesterification of spent coffee grounds oil in supercritical methanol

The process starts with weighing approximately 2 g of spent coffee. This sample is introduced in bed reactor a cylinder vessel with internal diameter of 20 mm and total length of 30 mm and closed with two metallic porous plates of 3 mm thickness each. The packed bed reactor is then introduced in the oven (Figure 2.1).

Following, the methanol is introduced in the reaction system before turning on the oven, to avoid the degradation of the spent coffee during heating. To avoid losses, the BPR valve (Tescom 27-1700) is gradually regulated as the temperature increases, since the pressure of methanol increases with temperature. When the desired temperature is reached, methanol is pumped into the circuit through the liquid pump (LCD, Type NSI-33R) with a flow rate of 0.5 mL/min.

The first experiment was done at 523 K and 15 MPa, the other experiences were conducted at temperatures of 553, 583 and 603K for pressures of 20, 25 and 30 MPa, respectively. A yellow/brown to dark brown viscous solution was obtained in the various traps, being each trap immersed in ice baths. Each trap is changed every 10 minutes during 50 minutes. The zero time of a experience begins when the first drop falls in the trap.

2.2.3Direct Transesterification of spent coffee grounds oil in supercritical methanol/CO_2 mixture

The process of direct transesterification of spent coffee grounds oil in supercritical methanol/CO_2 mixture begins the same way as the process described in point 2.2.2 and as shown in Figure 2.1. In this process, the carbon dioxide is introduced in the system before turning on the oven, to avoid the degradation of the spent coffee during heating. When the operating temperature is reached, CO_2 is pumped into the system through the HPLC pump (KNAUER, model K-501). Following the BPR (Tescom 27-1700) is gradually regulated until the operation pressure is reached.

When the conditions of operation are reached, methanol is injected into the circuit through a liquid pump (Gearmotor, Type NSI-33R), with a flow rate of 0.5 mL/min, which is in a molar proportion of 70-90% relatively to CO_2 (see appendix E).

The direct transesterification of spent coffee ground oil in supercritical methanol and CO_2 as co-solvent was conducted in the range of temperatures of 473-573 K, pressure range of 10-20 MPa and molar ratio CO_2/ MeOH range of 10-30%. Collection of samples in this method is similar to that described in point 2.2.2. Solutions obtained from the supercritical fluid reaction of the spent coffee were analysed by Gas Chromatography (GC), both qualitatively and quantitatively, for their content in methyl esters and triglycerides.

2.2.4Evaporation of methanol in the sample

After the experimental run, the five collected samples were weighed in an analytical balance (METTLER TOLEDO, model PJ12).

The evaporation of methanol from the samples is performed by bubbling air into the collected samples. The air stream first passes through a molecular sieves packed bed in order to remove the moisture from the air and then by a flow regulator (Parker Pneumatic, model P3D) that regulates the flow of air that goes directly into the sample.

After complete evaporation of methanol, the final sample is weighed. Next, the sample was dissolved in 5ml of a solution of hexane with squalane in a concentration of 300mg/L. Squalane was used as internal standard for the sample analysis. However, the sample was not completely dissolved in hexane because it has a polar and a non-polar fraction.

The non-polar fraction was transferred to another vial, followed by evaporation of residual hexane. The polar fraction was weighted in an analytical balance (METTLER TOLEDO, model PJ12) and dissolved in 5mL of Methanol.

2.3Sample analysis

The fatty acids methyl esters (FAME) and triglycerides content of extracted samples was determined by gas chromatography (GC) with on-Column injection. FAME can be of two types: saturated or unsaturated. If they are saturated they have the general formula $CH_3(CH_2)_nCO_2CH_3$ (Figure 2.3).

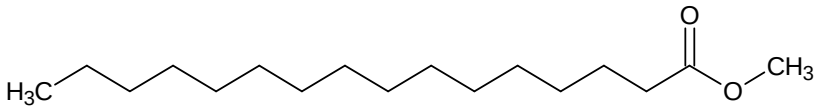

Figure 2.3 – Saturated fatty acid methyl ester: methyl palmitate.

If they are unsaturated, the difference is that they have double bonds along the carbon chain (Figure 2.4).

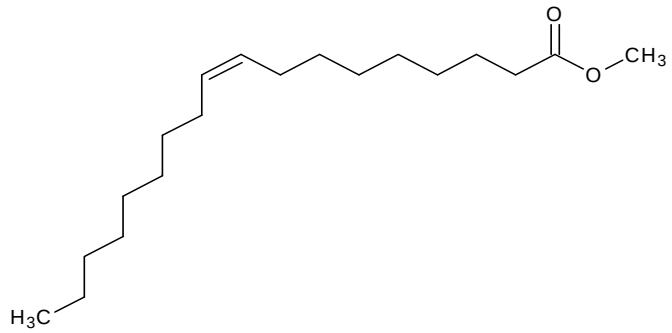

Figure 2.4 – Unsaturated fatty acid methyl ester: methyl oleate.

The non-polar sample in Hexane with Squalane (1:100 v/v) was used for on-column injection in chromatograph.

The chromatograph was a Trace GC 2000 Series with a flame ionization detector (FID); a ZB-5HT *Inferno* column, 10 m × 0.25 mm and 0.10 μm film thickness, from Zebron was used. This the column stationary phase being non-polar the methyl esters are separated due to their boiling points, and the unsaturated compounds are eluted before the corresponding saturated acids of the same chain length. For the samples under study, this column allows simultaneous determination of triglycerides and methyl esters in the sample. However, it was observed the occurrence of some overlapping of the methyl ester peaks corresponding to the methyl oleate, methyl stearate and methyl linoleate.

Hydrogen was used as carrier-gas .The flow starts with 0.1 $ml.min^{-1}$ during 1 minute and rises with the rate 1.5 $ml.min^{-1}.min^{-1}$ to 1.1 $ml.min^{-1}$ standing in this flow until the final of injection.

The oven temperature was 363–453 K at 15 $K{\cdot}min^{-1}$, followed by a ramp of 7 $K{\cdot}min^{-1}$ until 503 K and 30 $K{\cdot}min^{-1}$ until 653 K with a final holding time of 10 min. Peak identification was carried out using known standards and the software Excalibur.

Chapter 3 Results and Discussion

3.1 Direct transesterification in supercritical methanol

The direct transesterification of spent coffee ground oil in supercritical methanol was conducted in the range of temperatures of 523-603 K and pressures of 15-30 MPa. The critical temperature and pressure of methanol is 513 K and 8.1 MPa (Yin, Xiao and Song 2007).

To evaluate the effect of temperature and pressure in the transesterification of methyl esters using only methanol in the supercritical state, the curves presented in Figure 3.1 were obtained, which relate the yield of formation of methyl esters over time for the different conditions tested. These curves are built using the cumulative formation of methyl esters at each time of reaction, which means that when the curves reach a plateau, no more methyl esters are being formed and the reaction has reached its end. The yield of methyl esters formed was calculated taking into account the stoichiometry of the reaction and considering the percentage of oil contained in coffee ground to be 14% in weight. This percentage was obtained through soxhlet extraction with ethanol for the same spent coffee ground used in the various experimental runs. The experimental results are presented in table A-I, in appendix A. The methanol flow rate was kept constant in the whole set of experiments, at 0.5 ml/min.

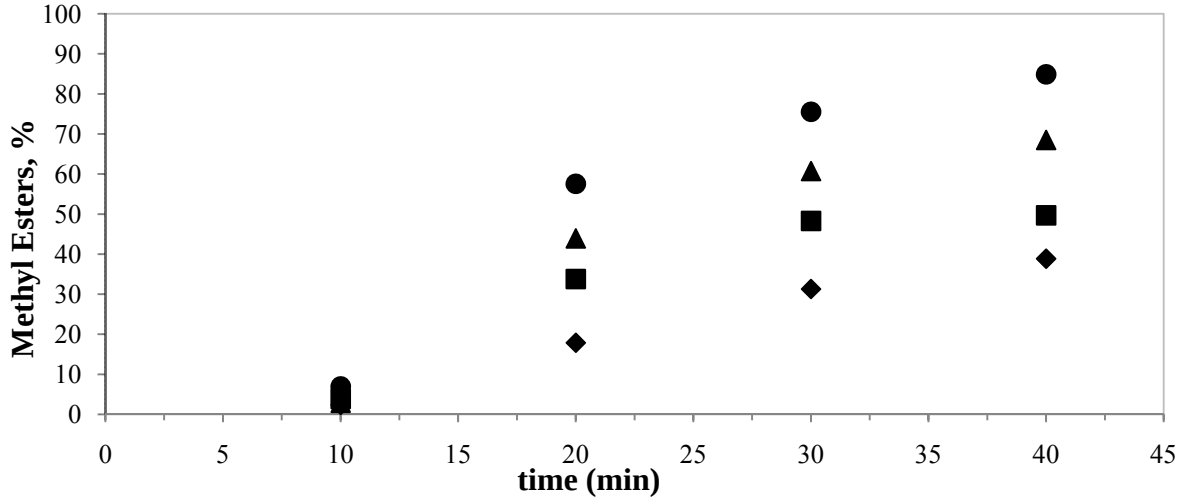

Figure 3.1 – Synthesis of spent coffee ground oil methyl esters, at various temperatures and pressures in supercritical methanol. ◆: 523K and 15 MPa; ■: 553 K and 20 MPa; ▲: 583 K and 25 MPa; ●: 603 K and 30 MPa. Methyl flow rate was 0.5 ml/min.

According with Figure 3.1, increasing temperature and pressure had a favorable influence on methyl ester yield. For experimental conditions of 603 K and 30 MPa, a high formation of methyl esters is obtained with a yield of 85% in 40 minutes.

The various samples have different yields of methyl esters and triglycerides. The amount of triglycerides extracted but not converted in different operating conditions is present in Figure 3.2. However, the yields of different methyl esters converted from the triglycerides extracted as a function of the operating conditions are indicated in Figure 3.3 (see also Table A-II in Appendix A). These figures show the total yields after 50 minutes of reaction, in the different conditions of pressure and temperature.

The triglycerides are not differentiated because there were no standards for all the triglycerides present in the oil of the spent coffee ground but only the tripalmitolein (C16:1) standard. This standard was used to build the calibration curve (see in Figure III–D in Appendix D) of triglycerides and to quantify all triglycerides in several samples. Methyl stearate, methyl oleate and methyl linoleate (C18) are presented together because the GC with injection on-Column could not separate the peaks.

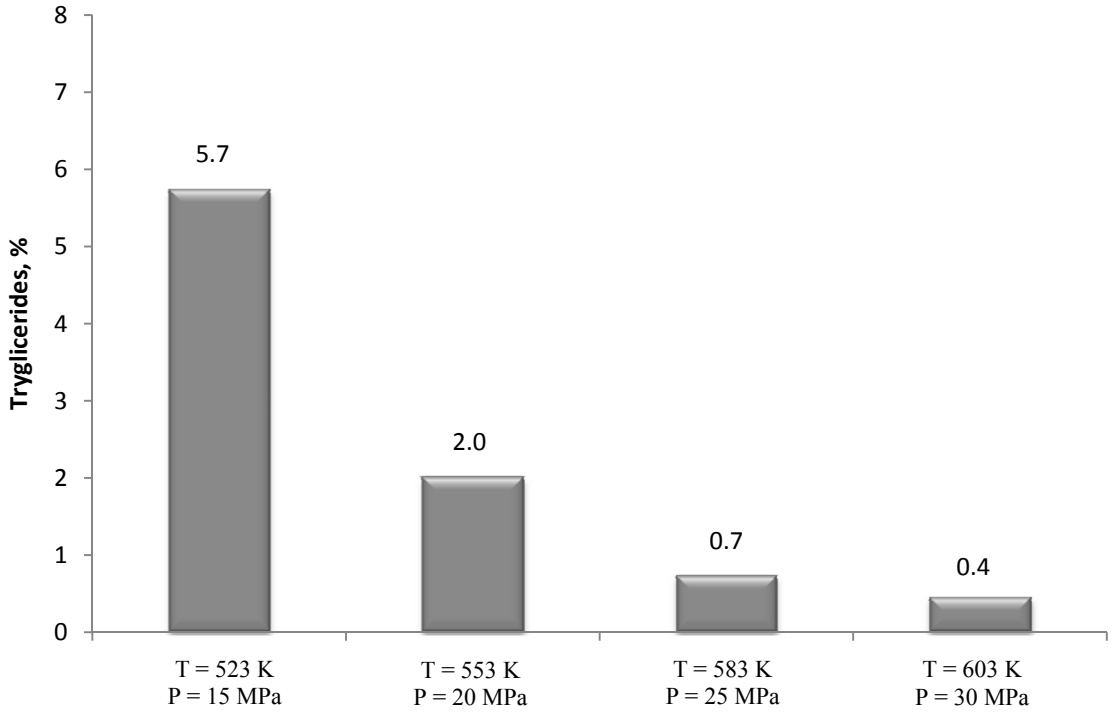

Figure 3.2 – Yield of Triglycerides extracted but not converted in supercritical transesterification with methanol for different conditions of pressure and temperature.

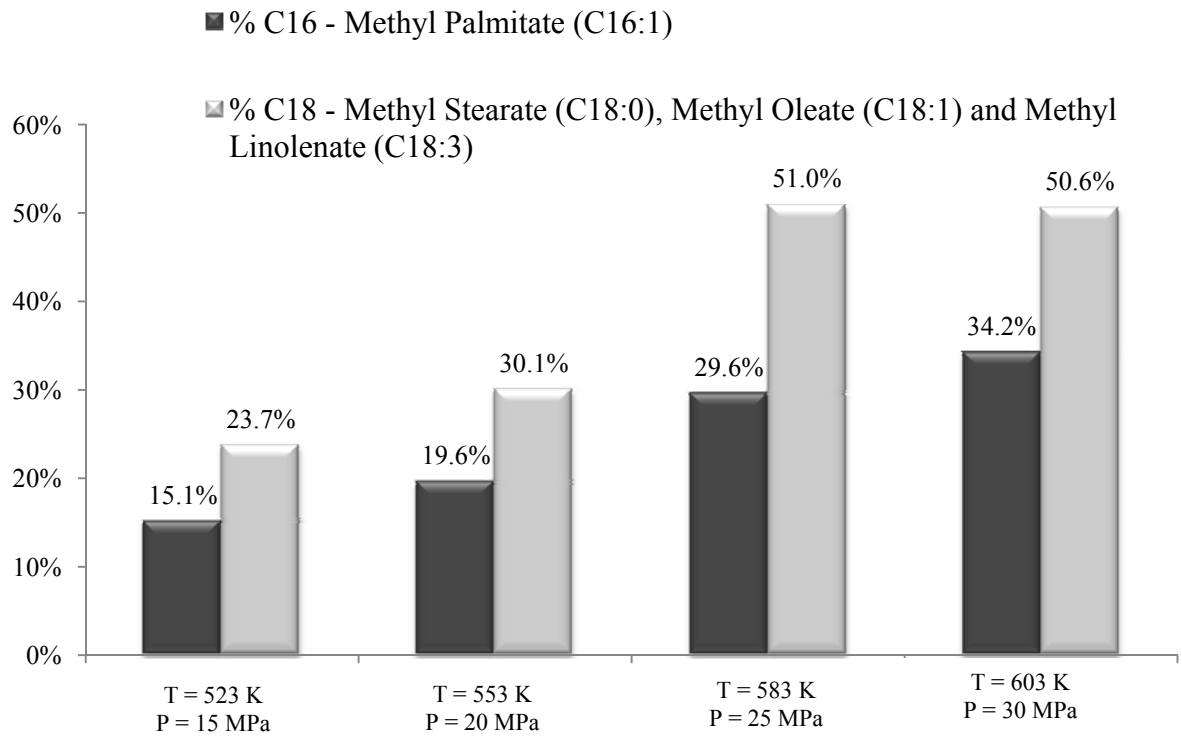

Figure 3.3 – Yield of methyl esters converted from triglycerides in supercritical methanol for different conditions of pressure and temperature.

Figure 3.3 show that methyl palmitate (C16) yield increases with increasing temperature and pressure. The same is true for C18, except for the highest temperature and pressure (583 K and 25 MPa), in which yield is constant at 51%.

These results are in accordance with Saka and Dadan (Saka and Dadan 2001), which show of that increasing the reaction temperature, especially for supercritical conditions, has a favorable influence on the yield of ester conversion and accelerates the reaction.

This occurs because high reaction yields could be expected when only the vapor phase is present and when the pressure and temperature correspond to a low density of supercritical methanol. Low density, supercritical methanol is characterized by a structure dominantly composed of the non-hydrogen bonded small species and a high density methanol is characterized by large molecular aggregates (Glisic, et al. 2007). Although the increase in pressure raises the density of supercritical methanol, this effect is offset by the increase of temperature because the raise in temperature decreases the density of supercritical methanol. However, as described in the section 2.2.2 of this thesis, in this process it is not possible to increase the temperature, while keeping the pressure constant.

After each experiment, methanol solvent is evaporated from samples using the method described in Chapter 2, section 2.2.3. This method results in the fractionation of each sample, in a polar and non polar phase. Figure 3.4 shows the mass fraction of polar and non-polar phases for each experimental condition.

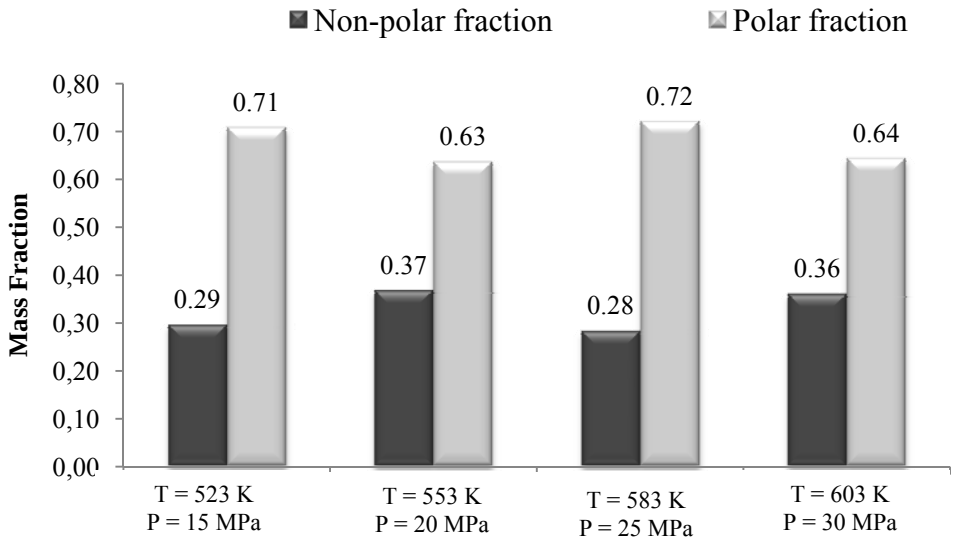

Figure 3.4 – Mass Fractions of polar and non-polar phases for several samples in direct transesterification with supercritical methanol.

It would be expected that the polar phase fraction increased with increasing temperature and pressure, but Figure 3.4 shows that the polar phase mass fractions are between 0.6 and 0.7 and non polar mass fractions are between 0.3 and 0.4 for the conditions tested. So, rotary evaporator would allow a better separation of the two phases but as the masses of the samples were very small, this method was not possible to be implemented. So the method applied (method described in chapter 2, section 2.2.3) was not the most efficient and the results shown in Figure 3.4 are not conclusive.

Comparing Figures 3.2 and 3.3 methyl esters yield increases and triglycerides yield decreases, with an increase of temperature and pressure. That is because the percentage of triglycerides that reacted increases with increasing temperature and pressure. Therefore, the percentage of triglycerides decreased and the methyl esters yield increases with increasing temperature. However the same is not true at lower temperatures and pressures, in this case the triglycerides yield is high and the methyl esters yield is lower.

3.2Direct transesterification from spent coffee grounds oil using supercritical methanol and CO_2 as co-solvent

The carbon dioxide as co-solvent is used to reduce pressure and temperature of operation. CO_2 is a good solvent for small and moderate organic molecules, and it is a low cost and facile material. CO2 has critical parameters of 304 K and 7.38 MPa, which is easy to achieve under the supercritical methanol condition (512 K, 8.09MPa). In order to verify the optimal point of the various factors that influence the transesterification with CO_2 as co-solvent in a minimum number of experiments, the design of experiments (DOE) was applied.

In the optimization of this extraction/transesterification process, three factors were considered:

1 – The reaction pressure;

2 – The molar ratio of methanol/CO_2;

3 – The reaction temperature.

All factors are changed at two levels (p=2), the lower level factor values are marked by the symbol "-1" and the upper ones by "+1". The conditions for each factor are shown in Table 3.1

Table 3.1 - Parameters studied to optimize of the direct transesterification of spent coffee ground oil using supercritical methanol and CO_2 as co-solvent, according to the DOE.

		Level	
Factor		**-1**	**+1**
1.	**pressure (MPa)**	10	20
2.	**Ratio CO_2/Methanol (%)**	10	30
3.	**Temperature (K)**	473	573

The design of experiments study includes eight design points (Table 3.2). The responses of all the eight runs in the analyzed example serve to determine the factor effects, with seven trials being independent possibilities of testing the effects and one serving for their comparison with the chosen fixed values (Lazic 2004). Design of experiments provides information about the interaction of factors and the way the total system works, something not obtainable through testing one factor at a time while holding other factors constant. Another advantage of DOE is that it shows how interconnected factors respond over a wide range of values, without requiring the testing of all possible values directly. That being so the DOE combined with statistical methods of data analysis, offers wider and more differentiated information on the system, while conclusions are of greater usability.

Table 3.2 - Design of experiments (DOE) of transesterification from spent coffee grounds oil using supercritical methanol and CO_2 as co-solvent.

Number of Trials	**Factor level combinations**				**Response**
	Levels	Pressure (MPa)	Ratio CO2/MeOH	Temperature (K)	%, Methyl Esters Formed
1	- - -	10	10%	473	4.06 %
2	+ - -	20	10%	473	6.36 %
3	- + -	10	30%	473	2.07 %
4	+ + -	20	30%	473	2.78 %
5	- - +	10	10%	573	93.44 %
6	+ - +	20	10%	573	62.33 %
7	- + +	10	30%	573	78.58 %
8	+ + +	20	30%	573	56.82 %

The procedure for extraction/transesterification process of spent coffee grounds with methanol and CO_2 as co-solvent is described in chapter 2 (Figure 2.1). The experimental results are presented in Figures 3.5 to 3.10 and in Table A – III and Table A-IV, in appendix A.

To evaluate the effect of temperature and pressure for a constant molar ratio CO_2/MeOH of 10% and 30%, the curves presented in Figures 3.5 an 3.6 were obtained.

These figures show the yield of formation of methyl esters over time for the different temperatures and pressures.

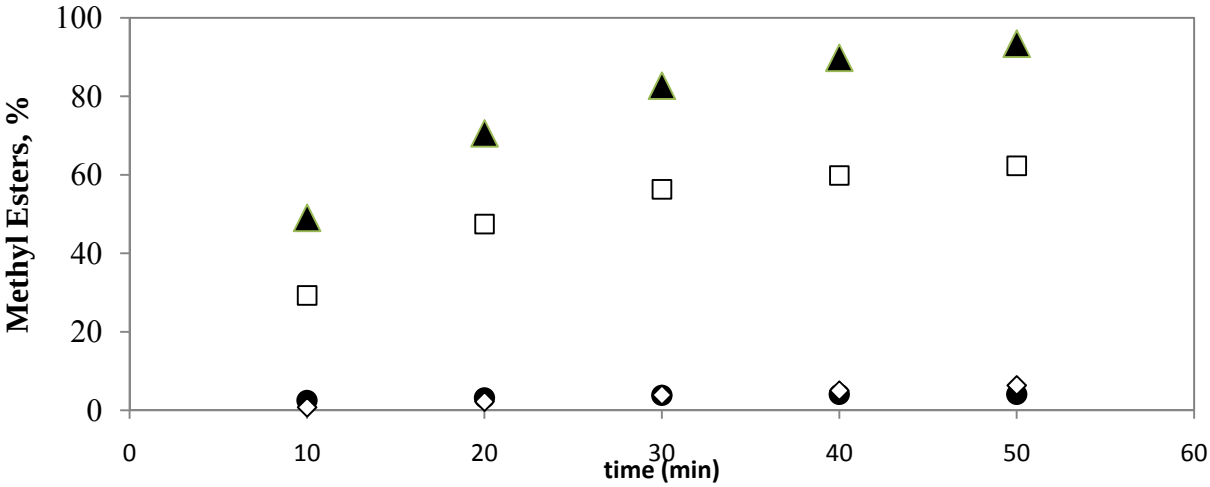

Figure 3.5 – Synthesis of spent coffee ground oil methyl esters, at various temperatures and pressures in supercritical methanol and CO_2 as co-solvent. Molar ratio CO2/MeOH was fixed at 10%. ●: 473 K and 10 MPa; ◇: 473 K and 20 MPa; ▲:573 K and 10 MPa; □:573 K and 20MPa.

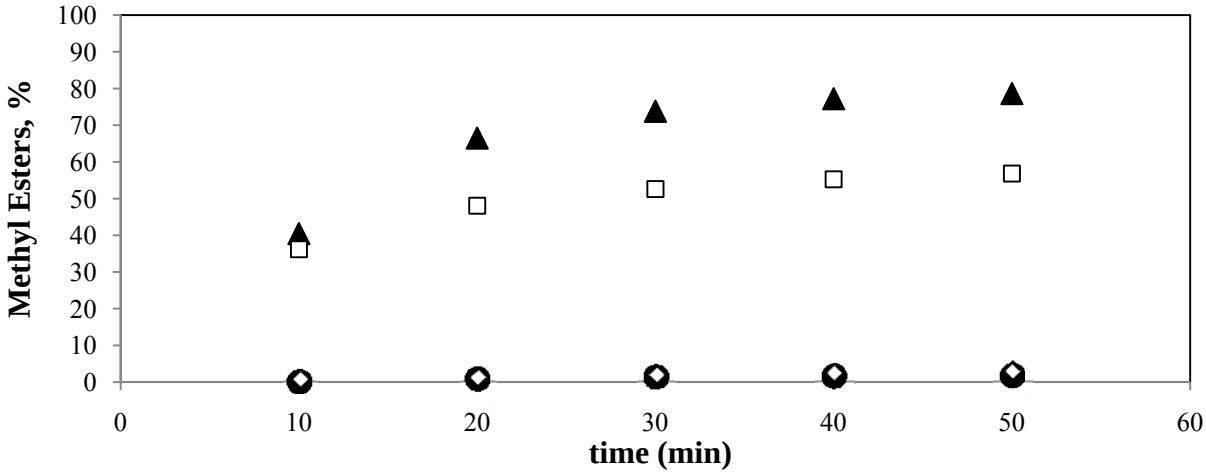

Figure 3.6 – Synthesis of spent coffee ground oil methyl esters, at various temperatures and pressures in supercritical methanol and CO_2 as co-solvent. Molar ratio CO2/MeOH was fixed at 30%. ●: 473 K and 10 MPa; ◇: 473 K and 20 MPa; ▲: 573 K and 10 MPa; □:573 K and 20 MPa.

The effect of the different temperatures and ratios CO_2/MeOH at a constant pressure can be observed through the Figures 3.7 and 3.8. The various experimental runs at a pressure of 10MPa are shown in Figure 3.7 and the experimental runs at a pressure of 20 MPa are shown in Figure 3.8.

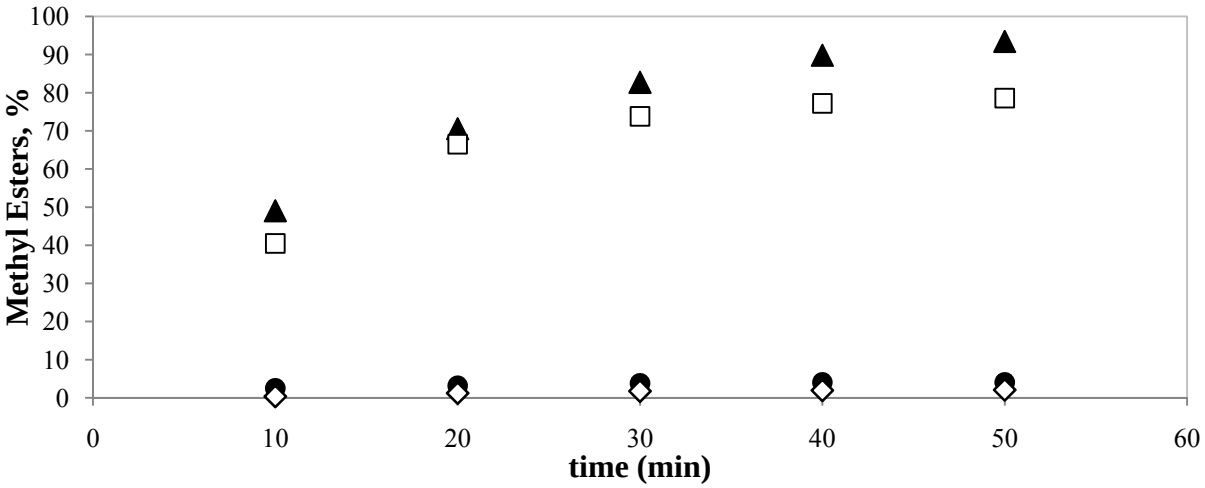

Figure 3.7 – Synthesis of spent coffee ground oil methyl esters, at various temperatures and ratios CO_2/MeOH in supercritical methanol and CO_2 as co-solvent. Pressure was fixed at 10MPa. ●: 473 K and 10%CO_2; ◇: 473 K and 30%CO_2; ▲:573 K and 10%CO_2; □:573 K and 30%CO_2.

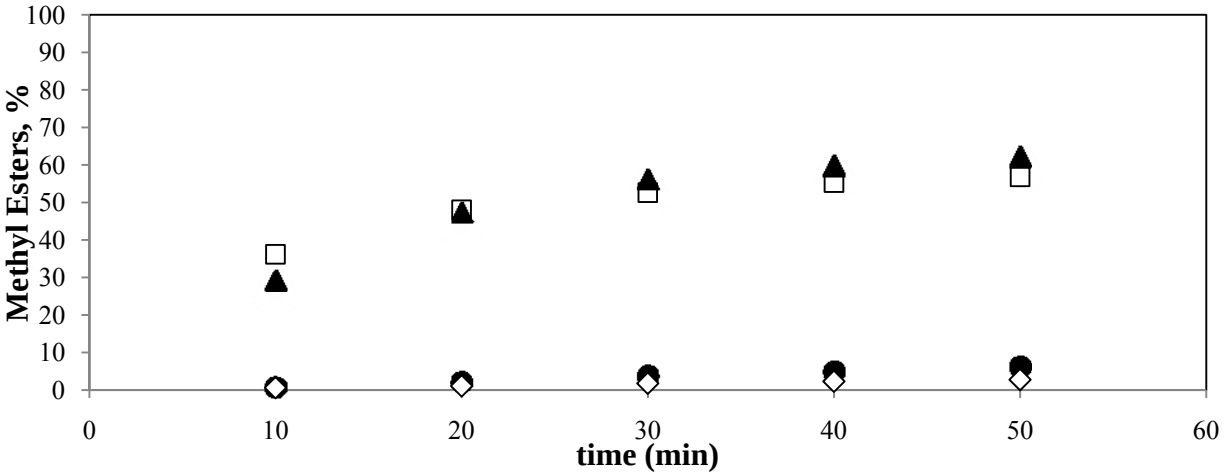

Figure 3.8 – Synthesis of spent coffee ground oil methyl esters, at various temperatures and ratios CO_2/MeOH in supercritical methanol and CO_2 as co-solvent. Pressure was fixed at 20MPa.: ●:473K and 10%CO_2; ◇:473K and 30%CO_2; ▲: 573K and 10%CO_2; □: 573K and 30%CO_2.

The effect of the different pressures and ratios of CO_2/MeOH at a constant temperature can be observed through Figures 3.9 and 3.10. The various experimental runs at a temperature of 473K are shown in Figure 3.9 and the experimental runs at a temperature of 573K are shown in Figure 3.10.

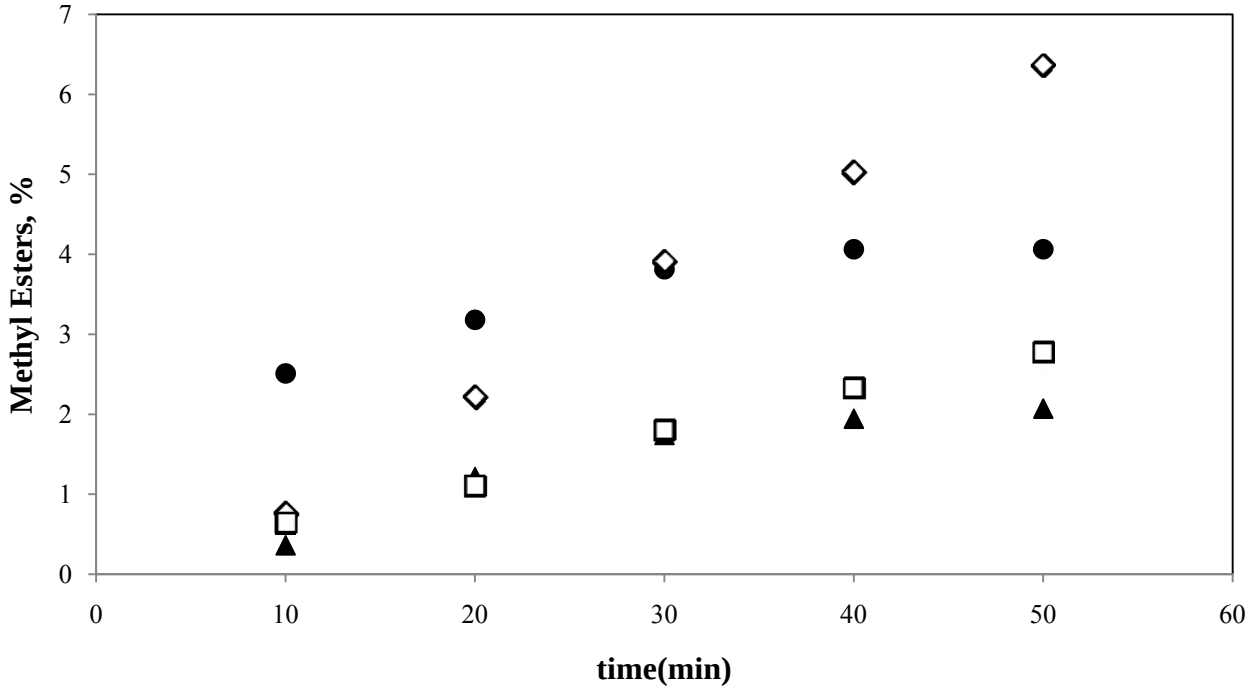

Figure 3.9 – Synthesis of spent coffee ground oil methyl esters, at various pressures and ratios CO_2/MeOH in supercritical methanol and CO_2 as co-solvent. Temperature was fixed at 473 K. ●: 10% CO_2 and 10MPa; ◇:10%CO_2 and 20MPa; ▲:30%CO_2 and 10MPa; □: 30%CO_2 and 20MPa.

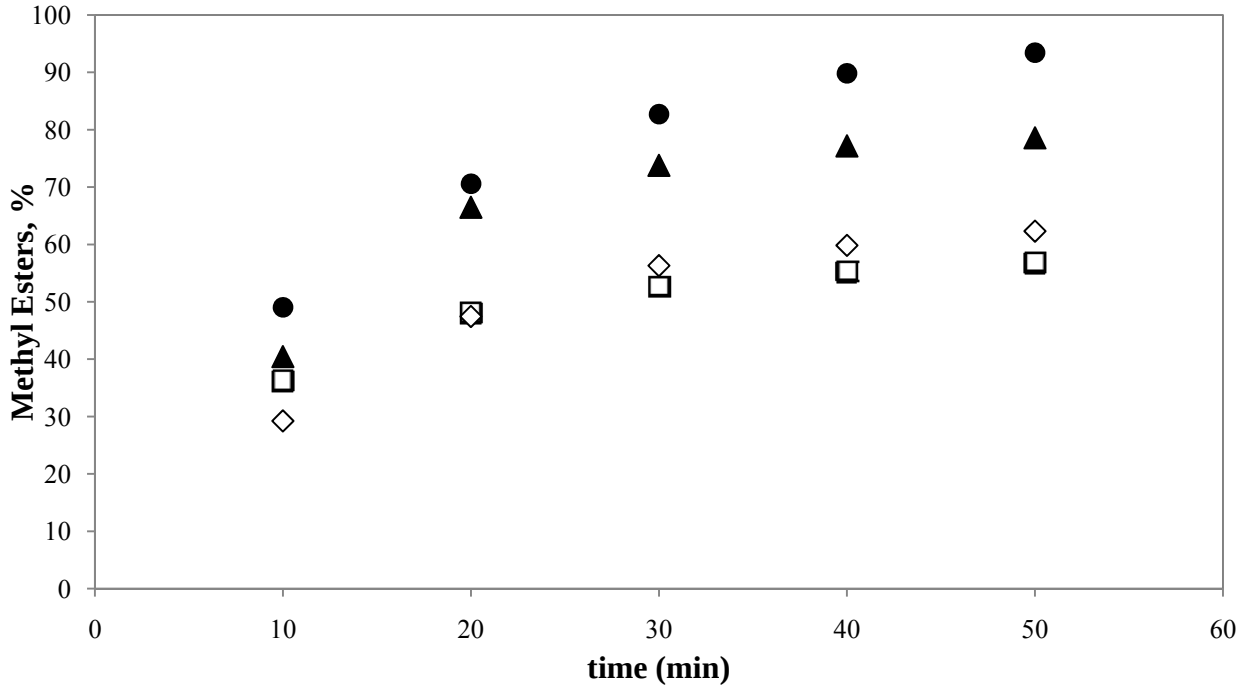

Figure 3.10 - Synthesis of spent coffee ground oil methyl esters, at various pressures and ratios CO_2/MeOH in supercritical methanol and CO_2 as co-solvent. Temperature was fixed at 573 K. ●: 10% CO_2 and 10MPa; ◇:10%CO_2 and 20MPa; ▲:30%CO_2 and 10MPa; □: 30%CO_2 and 20MPa.

Figures 3.5 to 3.6 shows that for supercritical methanol and CO_2 as co-solvent method, a molar ratio CO_2/MeOH of 10% can improve the methyl ester yield compared with the method where only supercritical methanol is used (Figure 3.1). This is because in the supercritical state, carbon dioxide behaves as a lipophilic solvent and so, the solution MeOH/CO_2 is able to extract more non polar solutes (Gupta and Shim 2006). Thus the addition of small concentrations of CO_2 as co-solvent may overcome the solubility problems of methanol and decrease the range of temperatures and pressures used to extract an equal or greater amount of triglycerides. Such fact was already observed by other authors (Yin, Xiao and Song 2007) (Han, Cao and Zhang 2005), who have shown that carbon dioxide added to the reaction mixture can decrease the operating temperature.

However, our data show that for a molar ratio of carbon dioxide/ methanol of 30%, the methyl esters yield decreased compared with the experimental data for 10% of ratio. According to Han et al. (Han, Cao and Zhang 2005) it was expected that gradually increasing the ratio of CO_2/MeOH, at the same conditions of temperature and pressure, the methyl esters yield would also increase until reaching a maximum. The results obtained in this work do not match that behavior. A greater number of experiments where the amount of carbon dioxide would vary are needed to draw better conclusions about this phenomenon. These experiments were not conducted due to shortage of time at the end of the experimental work.

The experimental results show that for the temperature of 473 K, the yields of methyl esters are relatively low. At a temperature of 573 K and a pressure of 20MPa or 10 MPa, the methyl esters yield is significantly higher in comparison with experimental runs at a temperature of 473K.

Figure 3.5 shows that at 573 K, 10 MPa and molar ratio carbon dioxide to methanol of 10%, yield of methyl esters exceeds 90% after 50 minutes and when the pressure is increased to 20MPa the yield decreases for 60%.

The influence of reaction pressure on supercritical transesterification has been investigated by some authors. Warabi et al (Warabi, Kusdiana and Saka 2004) and Bunyakiat et al. (Bunyakiat, et al. 2006) found that pressure did not significantly increase the reaction yield with high pressure. Their results differ from those obtained in this work.

The amount of triglycerides and methyl esters resulting from the direct transesterification with supercritical methanol and carbon dioxide as co-solvent as a function of the operating conditions are presented in Figures 3.11 and 3.12, respectively (see also Table A-V and Table A-VI in Appendix A). Results are presented in triglycerides and methyl esters percentage for each run with supercritical methanol and CO_2 as co-solvent at several temperatures, molar ratio CO_2/MeOH and pressures.

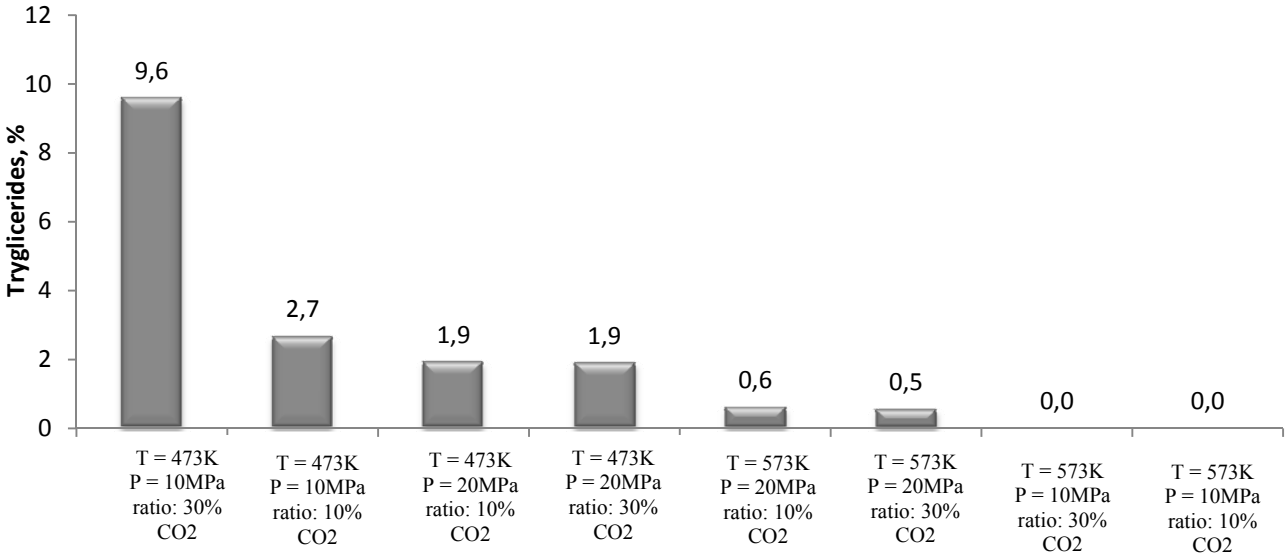

Figure 3.11 - Yield of Triglycerides extracted but not converted in supercritical transesterification with methanol and CO_2 as co-solvent in different condition of pressure and temperature.

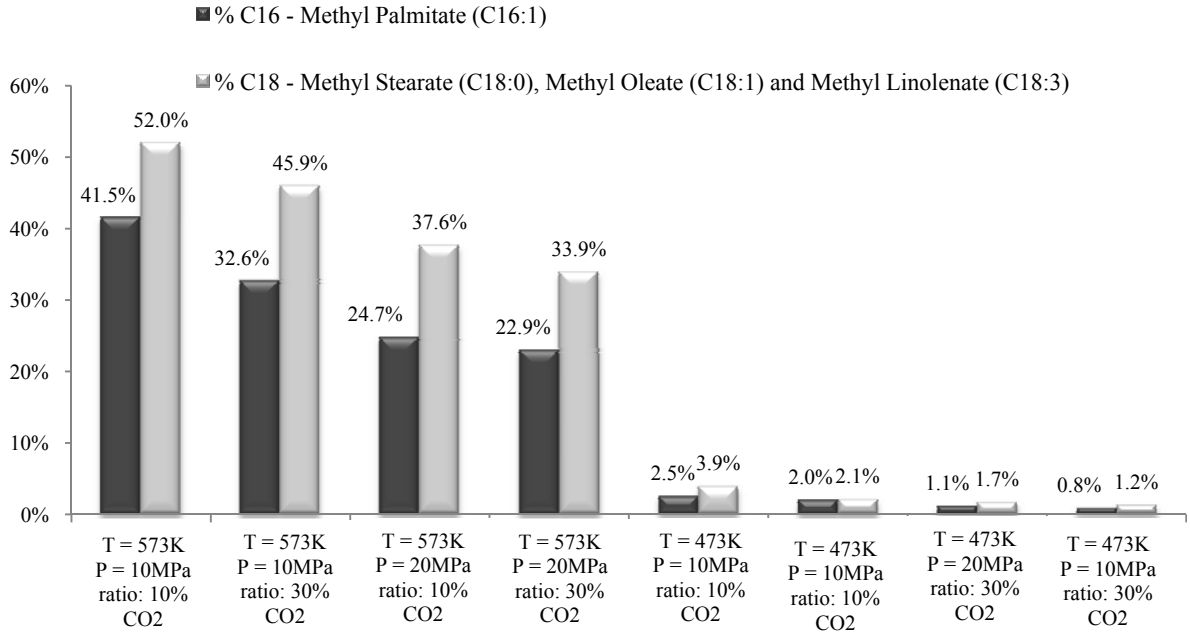

Figure 3.12 – Yield of methyl esters converted from triglycerides in supercritical transesterification with methanol and CO_2 as co-solvent in different condition of pressure and temperature.

Figure 3.12 shows that concentration of Methyl Palmitate (C16) increases with increasing temperature for the same conditions of pressure and ratio of CO_2/MeOH. The same is true for Methyl Stearate, Methyl Oleate and Methyl Linoleate (C18). In Figures 3.11 and 3.12, at the temperature of 473K, pressure of 10MPa and ratio of 30% CO_2, the methyl esters percentage (0.8% for C16 and 1.2% for C18) is very low, while the triglycerides percentage is high (9.6%). So, in these operating conditions the triglycerides were extracted in a high level but the percentage that reacted was very low. In the case where the temperature is 573K, the pressure is 10MPa and the ratio is 10% CO_2, the methyl esters percentage is very high and the triglycerides percentage is null. In this case, the yield in C18 considering the oil contained in coffee ground is 52%.

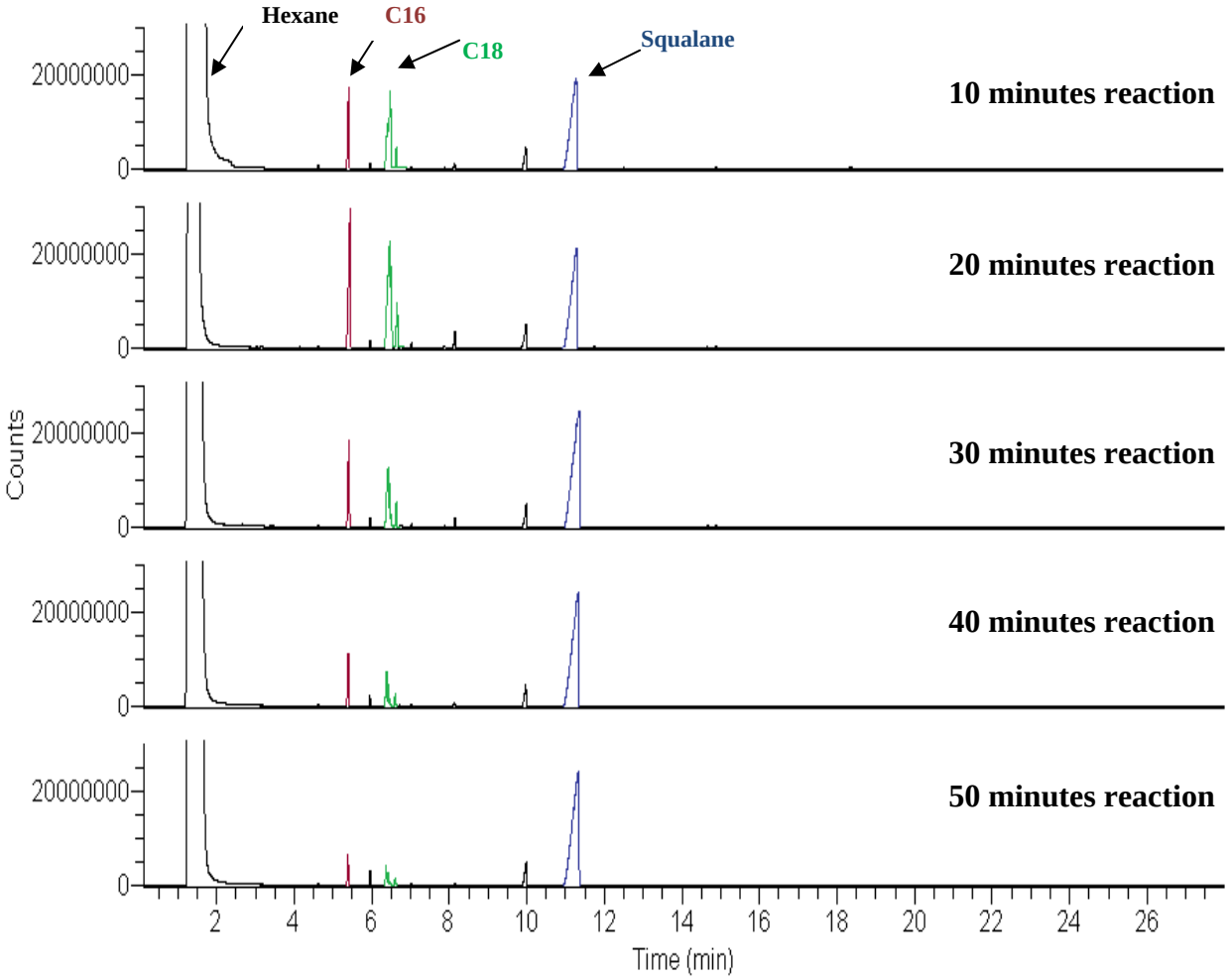

Figure 3.13 – Changes in GC chromatograms of the product as a function of reaction time in direct transesterification with supercritical methanol and co-solvent (molar ratio CO_2/MeOH is 0.1) at 573 K and 10 MPa. C16, methyl palmitate; C18 methyl stearate, methyl oleate and methyl linoleate.

Figure 3.13 shows the changes in GC chromatograms of product as a function of reaction time when spent coffee grounds is treated with supercritical methanol and carbon dioxide as co-solvent in molar ratio CO_2/MeOH of 10%, at 573 K and 10MPa. These correspond to the experimental conditions in where the yield of methyl esters was the highet, around 93% of the percentage of oil present in coffee. These chromatograms show that the amount of methyl esters increases in the sample up to reaction time of 20 minutes, but from 20 minutes the peaks of C16 and C18 decrease gradually. Figure 3.13 also shows that in these experimental conditions, samples do not present any triglycerides in different times of reaction, since the peaks of triglycerides appear from 18 minutes in GC chromatograms.

Figure 3.14 show the mass fraction of polar and non-polar parts for several experimental conditions. These fractions were obtained using the method described in chapter 2, section 2.2.3.

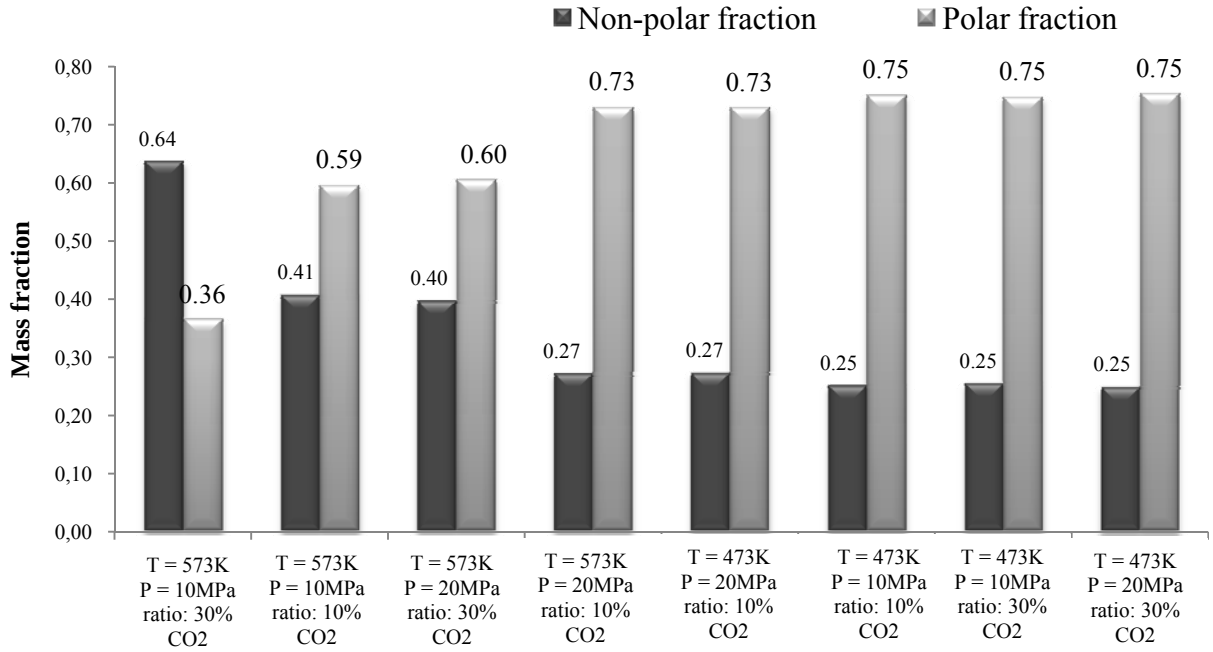

Figure 3.14 – Mass Fraction of polar and non-polar part for several samples in direct transesterification with supercritical methanol and CO_2 as co-solvent.

Figure 3.14 shows that for the temperature of 473 K, the non polar fraction and the polar fraction are in average approximately 0.25 and 0.75, respectively. The largest non polar fraction occurs at the temperature of 573 K, the only exception being 20MPa and molar ratio CO_2/MeOH of 10%, where the non polar fraction is 0.27. This figure also indicates that the temperature is the most significant factor but the influence of

operating variables can be better interpreted by ANOVA table (see Table I-B in Appendix B) given by software STATISTICA7. Analyzing this table, it appears that the temperature is the most significant factor in the methyl esters yield because the $F_{temperature}$=635.34 is higher than the $F_{critical}$=161.4.

Respond surface designs (Figure 3.15 to 3.17) were obtained using STATISTICA7 software and considering the responses of the design of experiences (Table 3.2). Response surface designs indicate the estimation of the different yields of methyl esters for several temperatures, pressures and ratios, given by STATISTICA7 software.

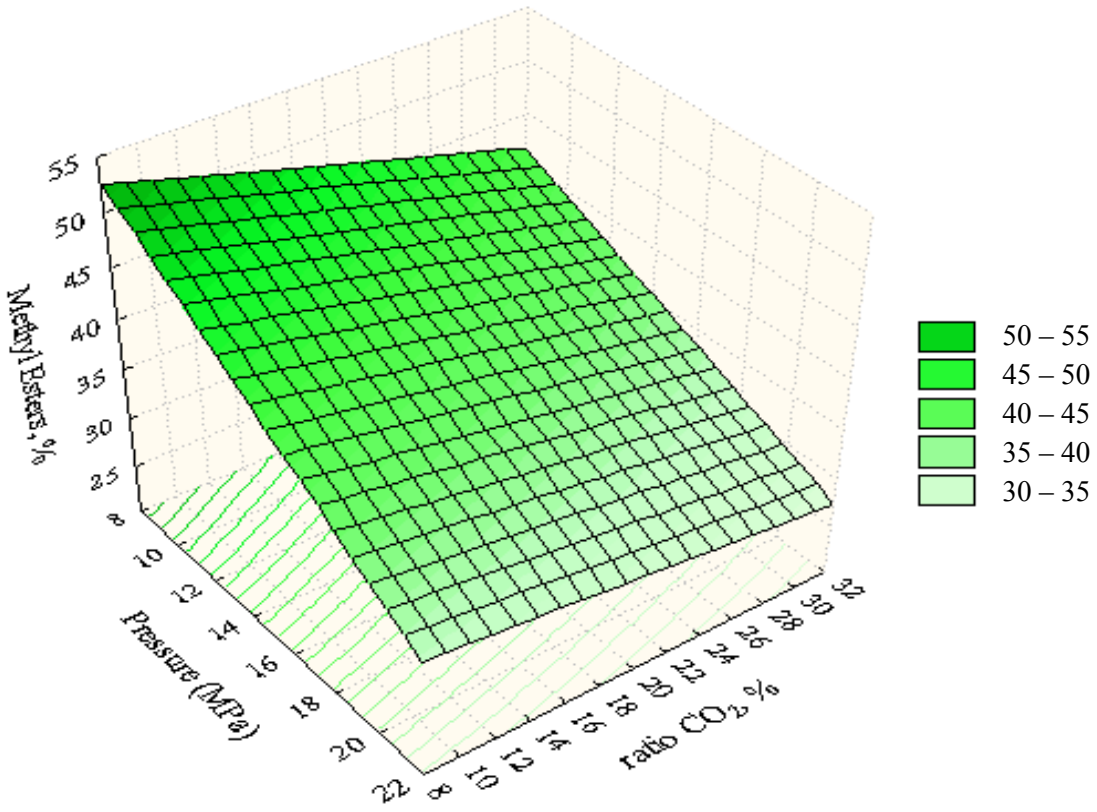

Figure 3.15 – Percentage of methyl esters estimated by STATISTICA7 software, varying the pressure and the ratio CO_2/MeOH, at a constant temperature of 523 K.

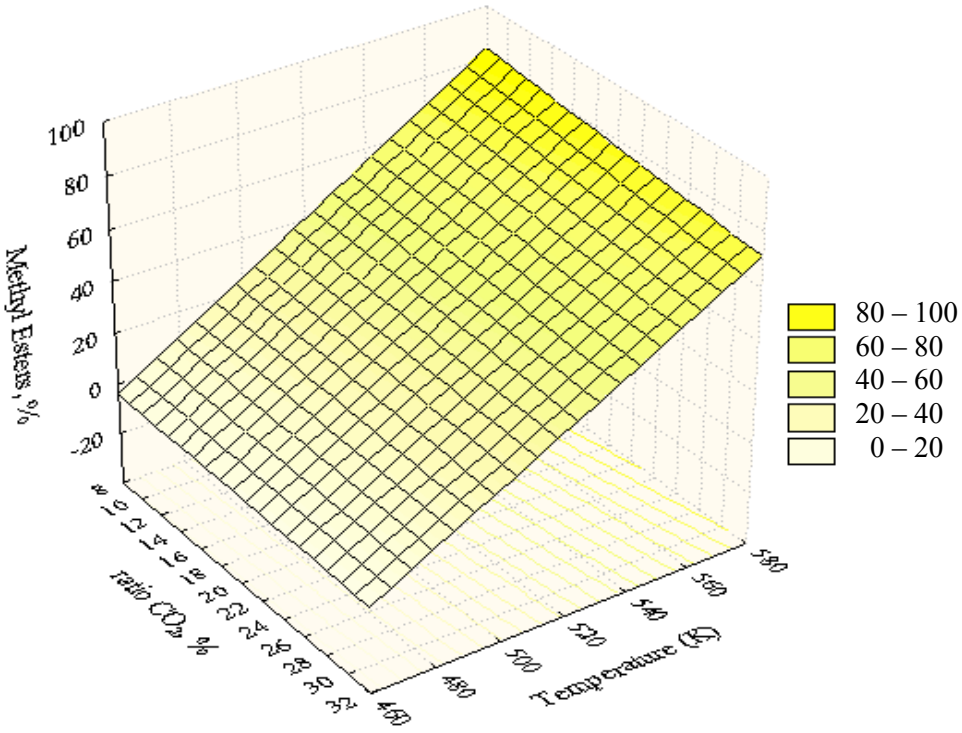

Figure 3.16 – Percentage of methyl esters estimated by STATISTICA7 software, varying the temperature and the ratio CO_2/MeOH, at a constant pressure of 15MPa.

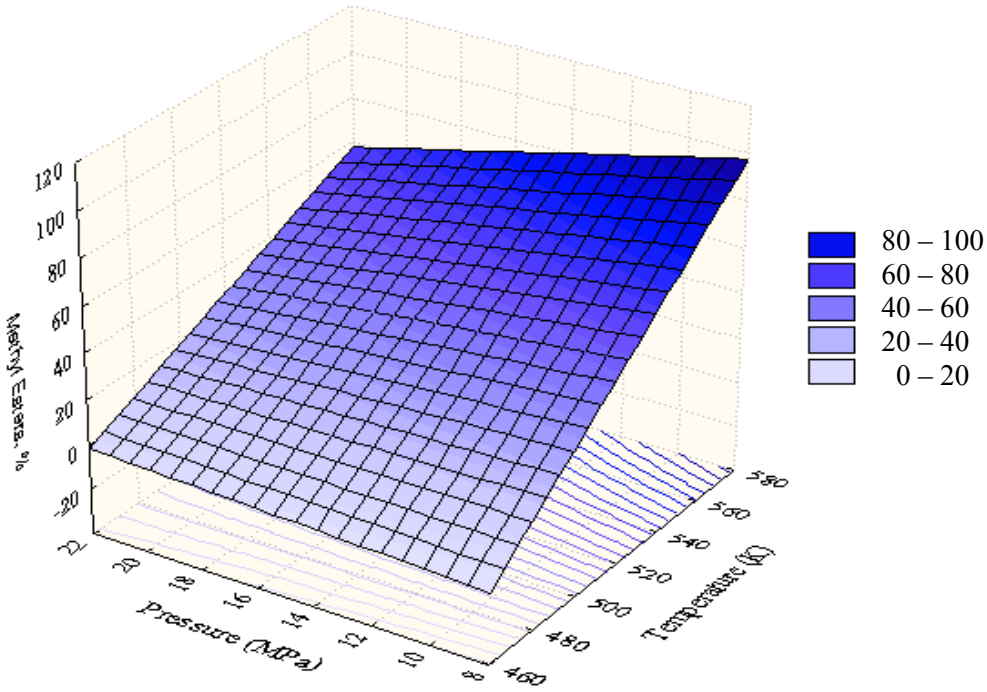

Figure 3.17 – Percentage of methyl esters estimated by STATISTICA7 software, varying the pressure and the temperature, at a constant ratio CO_2/methanol of 20%.

The figures above indicate that the best methyl esters yield occurs at higher temperature, lower pressure and lower ratio CO_2/MeOH of the range tested in design of experiments (DOE).

The validity of the experimental method has been confirmed by two repetitions of the best essay at 573 K, 10 MPa and molar ratio CO_2/MeOH of 10%. As can be seen from Figure 3.18, Table 3.3 and appendix C, both experiments have similar values of methyl esters yield, which accounts for the reproducibility of the experimental method.

Table 3.3 – Yield of methyl esters for the best level and two replications for a molar ratio CO_2/methanol of 10%, temperature of 573 K and pressure of 10 MPa.

Test	Methyl esters, %
Experimental test	93.43
Replica 1	93.40
Replica 2	93.44

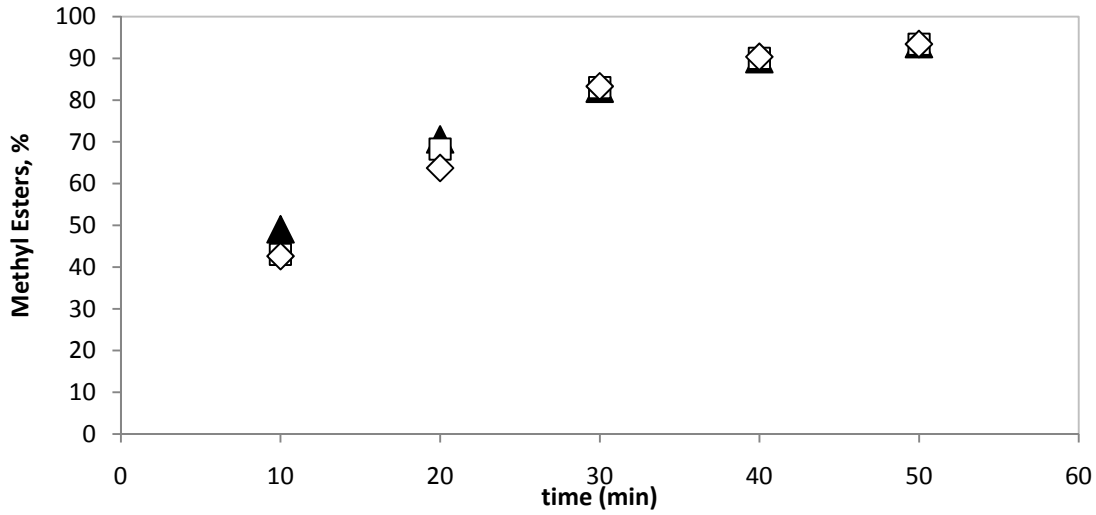

Figure 3.18 – Methyl Esters yield for the best essay at 573 K, 10 MPa and molar ratio CO_2/MeOH of 10% and two replications. ▲: Experimental test; □: Replica 1; ◇: Replica 2.

The validity of design of experiments has been confirmed by a experiment at 523 K, 10 MPa and molar ratio carbon dioxide to methanol of 10%.That being so the Figure 3.18 shows that the methyl esters yield increased with increase in temperature for a molar ratio CO_2/MeOH and pressure constants.

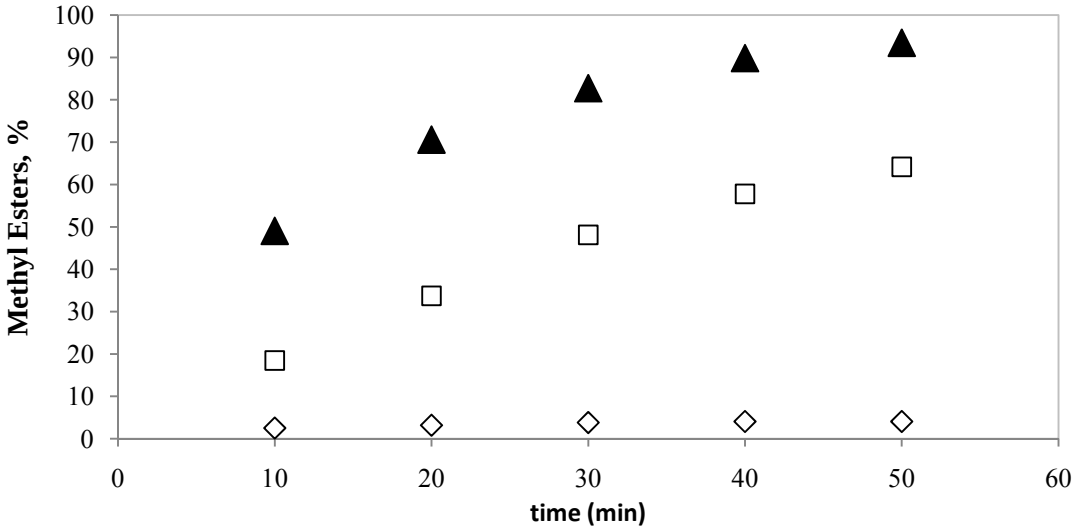

Figure 3.19 – Synthesis of spent coffee ground oil methyl esters, at various temperatures in supercritical methanol and CO_2 as co-solvent. Carbon dioxide and methanol were taken at a molar ratio of 10% with a constant pressure at 10 MPa. ◇: 473 K; □: 523 K; ▲:573 K.

The above figure shows that the methyl esters yield is approximately 60% at 523K, 10MPa and molar ratio CO_2/MeOH of 10%. However, Figure 3.15 shows that for the same operating conditions the estimated yield of methyl esters is approximately 53%. Thus, the experimental result show a variation in relation to the result estimated by the STATISTICA7 software and considering the responses of the design of experiences. This is because the number of tests used in this work is reduced, that is, it needs a greater number of experiments in order to obtain a better estimate. A great number of experiments were not made due to shortage of time at the end of the experimental time.

Chapter 4 Conclusions and Scope for Future Works

In recent years, biodiesel has become more attractive to replace petroleum fuel because of its environmental benefits. A sustainable biofuel has two favorable properties, which are availability from renewable raw material and its lower negative environmental impact than that of fossil fuels. However, the foremost problem that the biodiesel industry faces nowadays is the availability of low-cost and good quality feedstock. To meet this problem, industries use waste vegetable oil to produce low-cost biodiesel. That being so the spent coffee grounds can be a potential source for the production of biodiesel.

The basic idea behind this work was proven: extraction and transesterification with supercritical methanol from spent coffee grounds is possible in a single step. Spent coffee grounds oil was converted to methyl esters in supercritical methanol with yield of 85%. It was observed that increasing the reaction temperature and pressure, had a favorable influence the methyl esters yield. It was observed that coffee biodiesel consists of esters of palmitic acid, linolenic acid, oleic acid and stearic acid.

Supercritical methanol with a co-solvent process is superior to the conventional supercritical methanol method. As a co-solvent, CO_2 is both easy to add to the system beforehand and to remove from the system when the reaction is complete. The merit of this method is that lower reaction temperatures and pressures are required. Compared with the conventional supercritical methanol method, less energy process is required for a better yield of methyl esters. However, in order to determine the optimal conditions of the various factors that influence the transesterification with CO_2 in a minimum number of experiments, the design of experiments was applied. It was demonstrated that, at a reaction temperature of 573 K, a pressure of 10 MPa and a molar ratio CO_2/MeOH of 0.1, a 93% yield of methyl esters was observed.

The results obtained with this study suggest the importance of conducting further work to better understand the influence of different factors in the optimization process. Furthermore, the proposals are:

- ✓ Testing other operating conditions such as pressures below to 10 MPa or molar ratios CO_2/MeOH between 5 and 20%;

- ✓ To verify the obtained results and because the limitations of GC on-Colum, it is suggested an application of analytical methods such as HPLC;

- ✓ Biodiesel fuel prepared from spent coffee grounds could be analyzed by ASTM analysis. This analysis of the results would show the properties of biodiesel obtained from spent coffee grounds;

- ✓ Transesterification with supercritical methanol and carbon dioxide mixture from other waste sources, as waste cooking oil, could be tested;

- ✓ The experimental data obtained by doing the experiments should be modeled for direct transesterification with methanol/carbon dioxide mixtures so that the process can be scaled up for industrial use. The modeling of the reaction will determine the actual ability of spent coffee grounds on a commercial scale. Modeling of the extraction and reaction will also give a clear idea about the effect of different parameters.

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Appendix

Appendix A – Experimental results

Table A. I - Experimental data for direct transesterification of spent coffee grounds oil with supercritical methanol.

Temperature (K)		**523**	**553**	**583**	**603**
Pressure (MPa)		**15**	**20**	**25**	**30**
	Time (min)	**Mass (mg)**			
Methyl Esters formed(C16 and C18)	**10**	6.56	9.78	7.51	17.73
	20	42.75	76.39	106.99	129.07
	30	37.06	37.04	43.83	45.86
	40	20.85	3.62	20.26	23.82
	Total	**107.22**	**126.83**	**211.10**	**216.48**
C16 (Methyl palmitate)		39.41	47.29	73.12	82.57
C18 (Methyl stearate, methyl linoleate and methyl oleate)		67.82	79.54	137.98	133.91
Triglycerides		16.32	53.06	19.82	11.70
Initial mass of spent coffee grounds (g)		**2.03**	**1.88**	**1.93**	**1.88**
Mass of oil in spent coffee grounds (g)		**0.28**	**0.26**	**0.27**	**0.26**

Table A. II - Methyl Esters formed and triglycerides extracted mass in supercritical transesterification with methanol in different condition of pressure and temperature.

Temperature (ºC)	**523**	**553**	**583**	**603**
Pressure (bar)	**15**	**20**	**25**	**30**
C16	15.1%	19.6%	29.6%	34.2%
C18	23.7%	30.1%	51.0%	50.6%
Triglycerides	7.0%	2.4%	0.9%	0.5%

Table A. III - Experimental data for direct transesterification of spent coffee grounds oil with supercritical methanol and CO_2 as co-solvent at a molar ratio CO_2/methanol of the 10%.

Temperature (K)		**473**	**523**	**573**	**573**	**573**	**473**	**573**
Pressure (MPa)		**10**	**10**	**10**	**10**	**10**	**20**	**20**
					Replication1	**Replication2**		
Methyl Esters formed(C16 and C18)	**Time (min)**	**Mass (mg)**						
	10	6.52	55.39	136.34	121.50	133.84	2.34	90.01
	20	1.68	45.49	59.90	71.62	66.09	4.53	55.98
	30	1.65	43.29	33.79	41.57	61.45	5.26	27.25
	40	0.65	29.04	19.80	19.79	22.18	3.48	10.85
	50	-	19.26	10.08	9.50	9.56	4.16	7.62
	Total	**10.50**	**192.47**	**259.91**	**263.98**	**293.14**	**19.76**	**191.71**
C16 (Methyl palmitate)		4.90	78.11	109.48	94.38	113.51	7.29	71.84
C18 (Methyl stearate, methyl linoleate and methyl oleate)		5.60	114.36	150.42	169.60	179.63	3.98	119.87
Triglycerides		7.17	9.07	-	-	-	6.15	1.73
Initial mass of spent coffee grounds (g)		**1.92**	**2.22**	**2.05**	**2.08**	**2.31**	**2.29**	**2.12**
Mass of oil in spent coffee grounds (g)		**0.27**	**0.31**	**0.29**	**0.29**	**0.32**	**0.32**	**0.30**

Table A. IV - Experimental data for direct transesterification of spent coffee grounds oil with supercritical methanol and CO_2 as co-solvent at a molar ratio CO_2/methanol of the 30%.

Temperature (K)		**573**	**573**	**473**	**473**
Pressure (MPa)		**20**	**10**	**10**	**20**
Methyl Esters formed(C16 and C18)	**Time (min)**				
	10	108.30	121.42	1.12	2.06
	20	35.47	78.49	2.62	1.50
	30	13.61	22.08	1.62	2.26
	40	7.92	10.28	0.62	1.71
	50	4.67	4.22	0.39	1.45
	Total	**169.97**	**236.48**	**6.37**	**8.99**
C16 (Methyl palmitate)		64.90	93.00	2.39	3.42
C18 (Methyl stearate, methyl linoleate and methyl oleate)		105.07	143.47	3.98	5.57
Triglycerides		1.87	-	30.47	6.32
Initial mass of spent coffee grounds (g)		**1.92**	**2.22**	**2.05**	**2.12**
Mass of oil in spent coffee grounds (g)		**0.27**	**0.31**	**0.29**	**0.30**

Table A. V – Methyl Esters formed and triglycerides extracted percentage in supercritical transesterification with methanol and CO_2 as co-solvent in different condition of pressure and temperature, at a molar ratio CO_2/methanol of 10 %.

Temperature (K)	**473**	**523**	**573**	**573**	**573**	**473**	**573**
Pressure (MPa)	**10**	**10**	**10**	**10** Replication1	**10** Replication2	**20**	**20**
C16[1]	2.0%	27.5%	41.5%	35.4%	38.2%	2.5%	24.7%
C18[2]	2.1%	36.7%	52.0%	58.0%	55.2%	3.9%	37.6%
Triglycerides	3.2%	3.6%	0.0%	0.0%	0.0%	2.3%	0.7%

Table A. VI - Methyl Esters formed and triglycerides extracted percentage in supercritical transesterification with methanol and CO_2 as co-solvent in different condition of pressure and temperature, at a molar ratio CO_2/methanol of 30 %.

Temperature (K)	**573**	**573**	**473**	**473**
Pressure (MPa)	**20**	**10**	**10**	**20**
C16[1]	22.9%	32.6%	0.8%	1.1%
C18[2]	33.9%	45.9%	1.2%	1.7%
Triglycerides	0.7%	0.0%	11.7%	2.3%

[1] Methyl Palmitate (C16:1)
[2] Methyl Stearate (C18:0), Methyl Oleate (C18:1) and Methyl Linoleate (C18:3)

Appendix B – ANOVA table of the design of the experiences

Table B. I – ANOVA table given by STATISTICA7 software.

Factors	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-Value
(1) Pressure	310.80	1	310.80	20.75
(2) Ratio CO_2/MeOH	84.09	1	84.09	5.62
(3) Temperature	9514.92	1	9514.92	635.34
1 by 2	7.53	1	7.53	0.50
1 by 3	390.32	1	390.32	26.06
2 by 3	27.32	1	27.32	1.82
Error	14.98	1	14.976	
Total SS	***10349.96***	***7***		

The information about factor effects that is obtained from the partitioning of the total sum of squares is summarized in an ANOVA table. In this table, the first column, "factors," designates the partitioning of the response variability into the various components included in the statistical model. The second column of ANOVA table contains the sums of squares for various main effects and interactions, and the error components of the model.

The third column, "degrees of freedom," partitions the sample size into similar components that relate the amount of information obtained on each factor, the interaction, and the error term of the model. The number of error degrees of freedom is the number of factor–level combinations multiplied by one less than the number of repeat tests for each combination.

The fourth column, "mean squares," contains the respective sums of squares divided by their number of degrees of freedom. These statistics are used for forming the *F*-ratios in the next column, each effect and interaction mean square is divided by the error mean square. The significant effects are those where $F_0 > F_{Critical}$ (Mason, Gunst and Hess 2003). $F_{critical}=F\alpha;\nu_{factor};\nu_{error}=F_{0.05;1;1}=161.4$, this value is calculated for a value of alpha = 0.05, indicating a 95% confidence level, can be removed from the F-table, indicated below.

Table B. II – F Cumulative Probabilities: 95% confidence level (α = 0.05)

v_1 / v_2	1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	∞
1	161.4	199.5	215.7	224.6	230.2	234.0	236.8	238.9	240.5	241.9	243.9	245.9	248.0	249.1	250.1	251.1	252.2	253.3	254.3
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43	19.45	19.45	19.46	19.47	19.48	19.49	19.50
3	10.13	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79	8.74	8.70	8.66	8.64	8.62	8.59	8.57	8.55	8.53
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.91	5.86	5.80	5.77	5.75	5.72	5.69	5.66	5.63
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.68	4.62	4.56	4.53	4.50	4.46	4.43	4.40	4.36
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.00	3.94	3.87	3.84	3.81	3.77	3.74	3.70	3.67
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.57	3.51	3.44	3.41	3.38	3.34	3.30	3.27	3.23
8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.28	3.22	3.15	3.12	3.08	3.04	3.01	2.97	2.93
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.07	3.01	2.94	2.90	2.86	2.83	2.79	2.75	2.71
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.91	2.85	2.77	2.74	2.70	2.66	2.62	2.58	2.54
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85	2.79	2.72	2.65	2.61	2.57	2.53	2.49	2.45	2.40
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.69	2.62	2.54	2.51	2.47	2.43	2.38	2.34	2.30
13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67	2.60	2.53	2.46	2.42	2.38	2.34	2.30	2.25	2.21
14	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60	2.53	2.46	2.39	2.35	2.31	2.27	2.22	2.18	2.13
15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.48	2.40	2.33	2.29	2.25	2.20	2.16	2.11	2.07
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.42	2.35	2.28	2.24	2.19	2.15	2.11	2.06	2.01
17	4.45	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49	2.45	2.38	2.31	2.23	2.19	2.15	2.10	2.06	2.01	1.96
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41	2.34	2.27	2.19	2.15	2.11	2.06	2.02	1.97	1.92
19	4.38	3.52	3.13	2.90	2.74	2.63	2.54	2.48	2.42	2.38	2.31	2.23	2.16	2.11	2.07	2.03	1.98	1.93	1.88
20	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.28	2.20	2.12	2.08	2.04	1.99	1.95	1.90	1.84
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.25	2.18	2.10	2.05	2.01	1.96	1.92	1.87	1.81
22	4.30	3.44	3.05	2.82	2.66	2.55	2.46	2.40	2.34	2.30	2.23	2.15	2.07	2.03	1.98	1.94	1.89	1.84	1.78
23	4.28	3.42	3.03	2.80	2.64	2.53	2.44	2.37	2.32	2.27	2.20	2.13	2.05	2.01	1.96	1.91	1.86	1.81	1.76
24	4.26	3.40	3.01	2.78	2.62	2.51	2.42	2.36	2.30	2.25	2.18	2.11	2.03	1.98	1.94	1.89	1.84	1.79	1.73
25	4.24	3.39	2.99	2.76	2.60	2.49	2.40	2.34	2.28	2.24	2.16	2.09	2.01	1.96	1.92	1.87	1.82	1.77	1.71
26	4.23	3.37	2.98	2.74	2.59	2.47	2.39	2.32	2.27	2.22	2.15	2.07	1.99	1.95	1.90	1.85	1.80	1.75	1.69
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2.31	2.25	2.20	2.13	2.06	1.97	1.93	1.88	1.84	1.79	1.73	1.67
28	4.20	3.34	2.95	2.71	2.56	2.45	2.36	2.29	2.24	2.19	2.12	2.04	1.96	1.91	1.87	1.82	1.77	1.71	1.65
29	4.18	3.33	2.93	2.70	2.55	2.43	2.35	2.28	2.22	2.18	2.10	2.03	1.94	1.90	1.85	1.81	1.75	1.70	1.64
30	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	2.09	2.01	1.93	1.89	1.84	1.79	1.74	1.68	1.62
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.08	2.00	1.92	1.84	1.79	1.74	1.69	1.64	1.58	1.51
60	4.00	3.15	2.76	2.53	2.37	2.25	2.17	2.10	2.04	1.99	1.92	1.84	1.75	1.70	1.65	1.59	1.53	1.47	1.39
120	3.92	3.07	2.68	2.45	2.29	2.17	2.09	2.02	1.96	1.91	1.83	1.75	1.66	1.61	1.55	1.50	1.43	1.35	1.25
∞	3.84	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.88	1.83	1.75	1.67	1.57	1.52	1.46	1.39	1.32	1.22	1.00

Source: (Mason, Gunst and Hess 2003)

Appendix C – Replication of the best level

Table C I – Yield of methyl esters for the best level and its two replications. Molar ratio CO_2/methanol of 10%, temperature of 573K and pressure of 10MPa.

Test	Methyl esters, %
Experimental test	93.43
Replication 1	93.40
Replication 2	93.44

$$Average = \bar{x} = \frac{1}{N}\sum_{i=1}^{N} x_i = \frac{x_{1+}\, x_{2+}\, x_{2+} \cdots + x_N}{N}$$

$$\bar{x} = \frac{93.43\% + 93.40\% + 93.44\%}{3}$$

$$\bar{x} = 93.42\%$$

$$Variance = \sigma^2 = \frac{1}{N}\sum_{i=1}^{N} (x_i - \bar{x})^2$$

$$\sigma^2 = \frac{1}{2}((93.43 - 93.42)^2 + (93.40 - 93.42)^2 + (93.44 - 93.42)^2)$$

$$\sigma^2 = 0.01\%$$

Appendix D – Calibration curves

Here, it is shown the calibration curves used to analyze the methyl esters and triglycerides in the various experimental runs. Squalane was used as internal standard with concentration of 300mg/L.

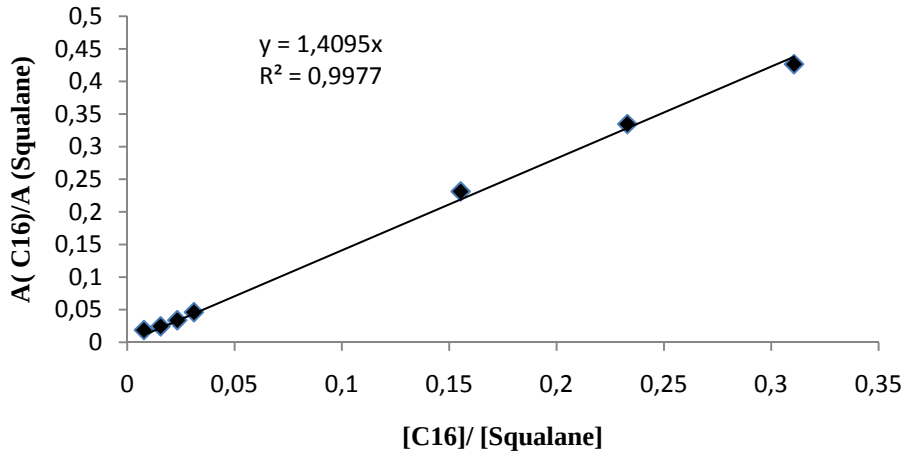

Figure D. I – Calibration curve for Methyl Palmitate (C16) peaks in GC, equation of calculated trend line and its correlation coefficient. A(C16): peak area of Methyl Palmitate.

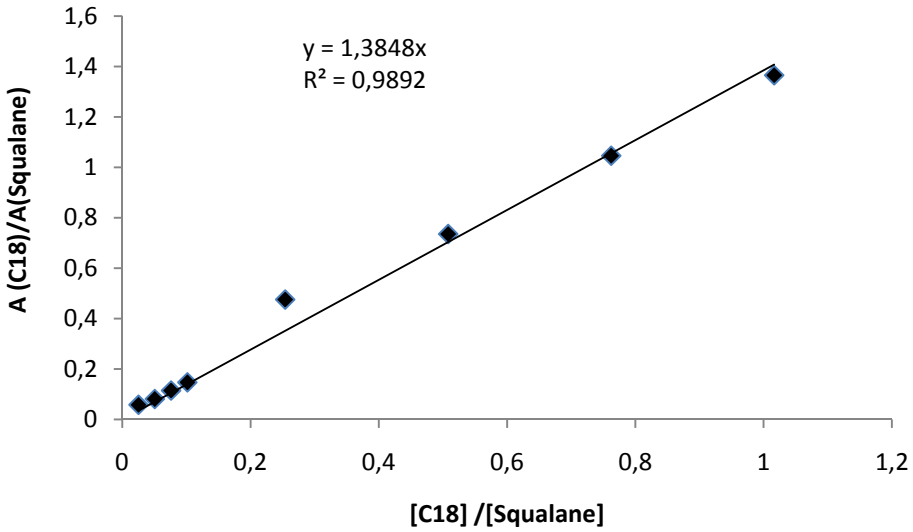

Figure D. II – Calibration curve for Methyl Stearate, Methyl Oleate and Methyl Linoleate (C18) peaks in GC, equation of calculated trend line and its correlation coefficient. A(C18): peak area of Methyl Stearate, Methyl Oleate and Methyl Linoleate.

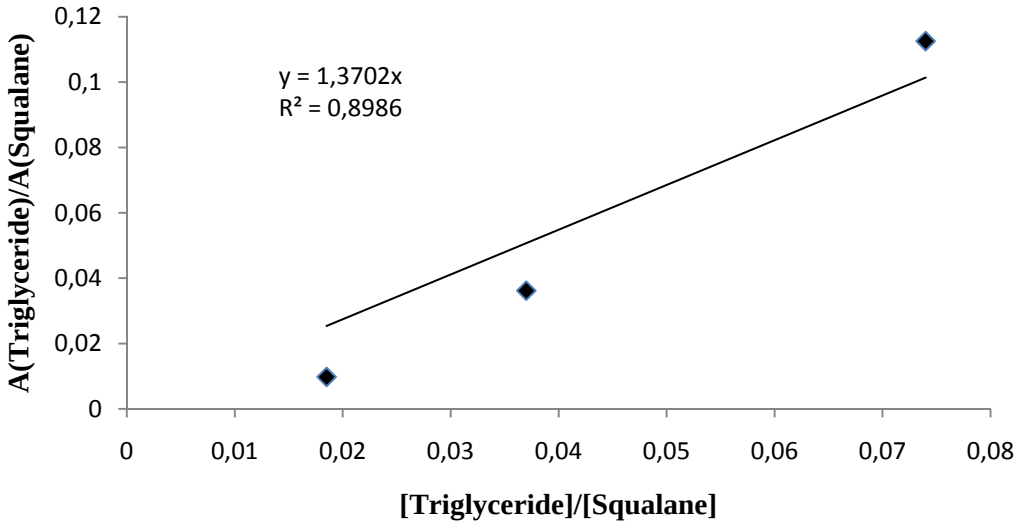

Figure D. III – Calibration curve for Tripalmitolein peaks in GC, equation of calculated trend line and its correlation coefficient. A(Triglycerides): peak area of Tripalmitolein.

Appendix E – Molar Ratios CO_2/MeOH

Table E I – Isobaric Properties for Methanol at 10 MPa.[1]

Temperature (K)	Pressure (MPa)	Density (g/ml)	Phase
288	10	0.80452	liquid
289	10	0.80363	liquid
290	10	0.80274	liquid
291	10	0.80185	liquid
292	10	0.80096	liquid
293	**10**	**0.80007**	**liquid**
294	10	0.79918	liquid
295	10	0.79829	liquid
296	10	0.79740	liquid
297	10	0.79651	liquid
298	10	0.79562	liquid

Table E II – Isobaric Properties for CO_2 at 10MPa.

Temperature (K)	Pressure (MPa)	Density (g/ml)	Phase
268	10	0.99825	liquid
269	10	0.99352	liquid
270	10	0.98874	liquid
271	10	0.98390	liquid
272	10	0.97901	liquid
273	10	0.97405	liquid
274	10	0.96903	liquid
275	10	0.96394	liquid
276	10	0.95879	liquid
277	**10**	**0.95356**	**liquid**
278	10	0.94825	liquid

Table E III – Isobaric Properties for Methanol at 20 MPa.[1]

Temperature (K)	Pressure (MPa)	Density (g/ml)	Phase
288	20	0.81262	liquid
289	20	0.81177	liquid
290	20	0.81092	liquid
291	20	0.81006	liquid
292	20	0.80921	liquid
293	**20**	**0.80836**	**liquid**
294	20	0.80751	liquid
295	20	0.80666	liquid
296	20	0.80581	liquid
297	20	0.80496	liquid
298	20	0.80411	liquid

[1] Source:webbock.nist.gov

Table E IV – Isobaric Properties for CO_2 at 20MPa.[1]

Temperature (K)	Pressure (MPa)	Density (g/ml)	Phase
268	20	1.0398	liquid
269	20	1.0359	liquid
270	20	1.0321	liquid
271	20	1.0283	liquid
272	20	1.0244	liquid
273	20	1.0205	liquid
274	20	1.0166	liquid
275	20	1.0126	liquid
276	20	1.0087	liquid
277	**20**	**1.0047**	**liquid**
278	20	1.0007	liquid

M_{MeOH} = 32 g/mol

M_{CO2} = 44 g/mol

Volume Flow (MeOH) = 0.5 ml/min

Volume Flow (CO_2) = 0.06 ml/min for ratio of 10%

Volume Flow (CO_2) = 0.17 ml/min for ratio of 30%

$$Molar\ ratio\ {}_{CO_2}/_{MeOH} = \frac{\frac{\rho_{CO_2} \times V_{CO_2}}{M_{CO_2}}}{\frac{\rho_{MeOH} \times V_{MeOH}}{M_{MeOH}}}$$

$$Molar\ ratio\ {}_{CO_2}/_{MeOH} = \frac{\frac{0.95356 \times 0.06}{44}}{\frac{0.80007 \times 0.5}{32}} = \frac{0.0013}{0.0125} = 0.104 = 10\%$$

$$Molar\ ratio\ {}_{CO_2}/_{MeOH} = \frac{\frac{0.95356 \times 0.17}{44}}{\frac{0.80007 \times 0.5}{32}} = \frac{0.0037}{0.0125} = 0.296 = 30\%$$

The temperatures considered for the calculations of molar ratios were 20 and 4 for the MeOH and CO_2, respectively. It was considered these temperatures because these were verified, in the average, in most experiments.

[1] Source:webbock.nist.gov